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Synthesis of Enantioenriched Piperidine-Based, Spirocyclic Analogues of AF64A and Acetylcholine

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SYNTHESIS OF ENANTIOENRICHED PIPERIDINE-BASED, SPIROCYCLIC ANALOGUES OF AF64A AND ACETYLCHOLINE

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LIST OF ABBREVIATIONS

Å	anstrom (10^{-8} cm)
Ac	acetyl
Acetyl-CoA	acetyl-coenzyme A
ACh	acetylcholine
AChE	acetylcholinesterase
AChM	acetylcholine mustard
AChR	acetylcholine receptor
AcO	acetoxy = $CH_3C(O)$-
Ac_2O	acetic anhydride
ACTH	adrenocorticotropin
ACTM	2-acetoxycyclopropyltrimethyl ammonium iodide
AF64A	ethylcholine mustard aziridinium (named after Dr. Abraham Fisher)
Anal	analysis
aq	aqueous
i-Bu	isobutyl
n-Bu	normal butyl
calcd	calculated
°C	degrees in Celsius
Cbz	carbobenzyloxy
ChAT	choline acetyl transferase
cm	centimeter(s)
CNS	central nervous system
δ	chemical shift in parts per million
D	dimensional
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
DMAP	N,N-dimethylaminopyridine
DPMSCl	diphenylmethylsilyl chloride

dt	doublet of triplet
ECMA	ethylcholine mustard aziridinium
equiv	equivalent
Et	ethyl
FT	Fourier Transform
GABA	*gamma*-aminobutyric acid
GC	gas chromatography
g	gram(s)
h	hour(s)
His	histidine
Hz	hertz
IR	infrared
J	coupling constant (NMR) in hertz
m	multiplet
Me	methyl
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
M	moles per liter
MHz	megahertz
mp	melting point
NaOH	sodium hydroxide
NMR	Nuclear Magnetic Resonance
Pd/C	palladium on activated carbon
ppm	parts per million
i-Pr	isopropyl
n-Pr	normal propyl
psi	pound per square inch
pyr	pyridine
q	quartet
(R)	*rectus*, Latin; right
R_f	retention factor

s	singlet
(S)	*sinister*, Latin; left
Ser	serine
t	triplet
TBDMSCl	*tert*-butyldimethylsilyl chloride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSCl	trimethylsilyl chloride
tol	toluene
Ts	*para*-toluenesulfonyl (tosyl)
t_R	retention time
3°	tertiary
Δ	reflux

To Mom and Dad, and memory of Brother

CHAPTER I

INTRODUCTION

A. Neurotransmission and Dementia

1. *Neurotransmission in Synapses*

Neuronal systems can be quite complex regulating an array of physiological functions through the interaction of sequentially coupled nerve cells that use different neurotransmitters. A neurotransmitter (a chemical messenger released from neurons into a synapse) can be excitatory in one system but inhibitory in another. For example, sympathetic ganglia have been shown to contain three kinds of receptors, as shown in the schematic diagram in Fig. 1.

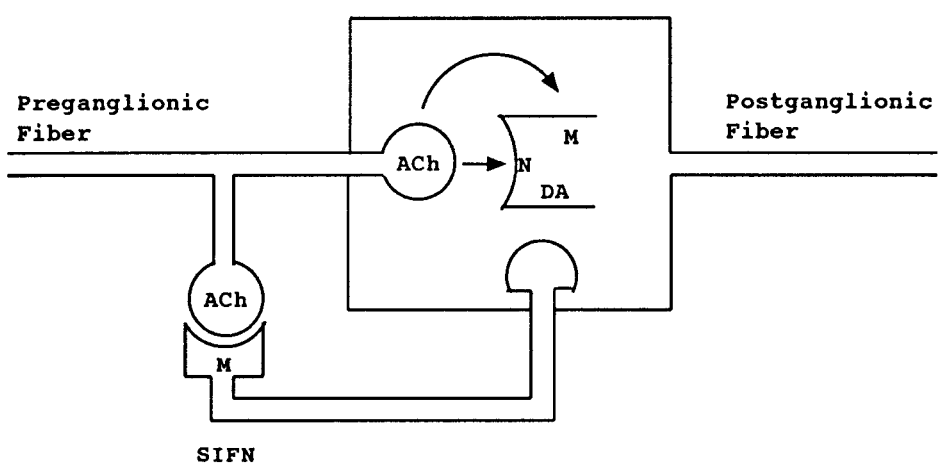


Figure 1. Interaction between neurons in a sympathetic ganglion. DA, dopaminergic receptor; M, muscarinic cholinergic receptor; N, nicotinic cholinergic receptor; ACh, acetylcholine; SIFN, small intensely fluorescing interneuron (Ryall, 1979).

Sympathetic ganglia, which normally operate on a cholinergic mechanism, also include small, intensely fluorescing neurons (SIFN) that produce dopamine and hyperpolarize the postganglionic neuron, thereby establishing a complex control system. A preliminary understanding of the sequential coupling of neurons now permits a discussion of the treatment of pathological states due to neuronal dysfunction. As an example, Fig. 2 briefly diagrams the pathophysiology of Parkinson's disease, a degenerative syndrome characterized by neuromotor disorders such as tremor, rigidity, a stooped posture, and difficulty in initiation and stopping movement.

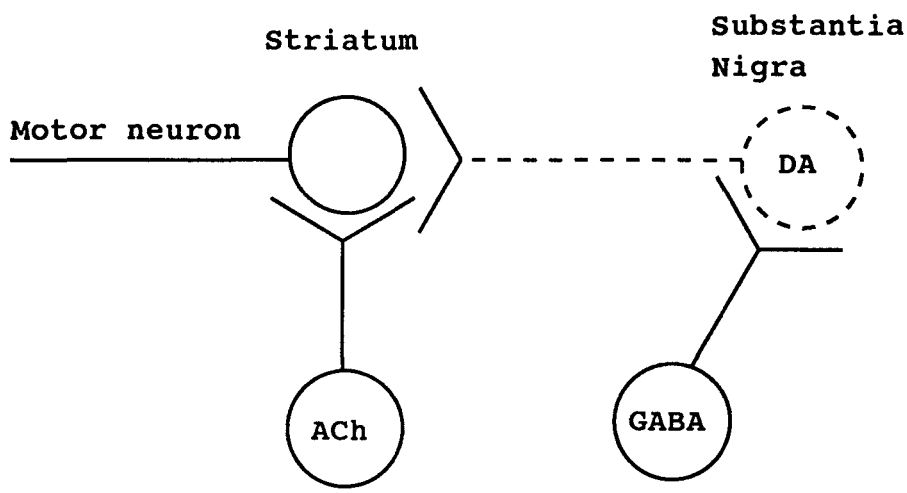


Figure 2. Neuronal networks. The motor neuron in the striatum is regulated by an excitatory cholinergic neuron and an inhibitory dopaminergic neuron, which in turn is influenced by a GABAergic fiber. Loss of the dopaminergic nigrostriatal fibers leads to excessive cholinergic stimulation of the motor neuron, resulting in Parkinson's disease.

Parkinson's syndrome results from the disappearance of dopaminergic neurons connecting the substantia nigra with the striatum. The loss of dopaminergic inhibition in the striatum causes cholinergic hyperactivity in this brain region, resulting in many of the neuromotor symptoms of the disease. Representative drug treatment for Parkinson's disease, therefore, consists of a combination of dopamine replacement and release facilitation therapies, as well as the use of cholinergic blocking agents to control parasympathetic hyperactivity.

2. *Drug Development for Senile Cognitive Decline*

The treatment of senile cognitive decline is one of the greatest challenges for the mental health sciences today. No truly effective therapy has yet been launched although research in the cognitive sciences has the potential to produce enormous personal, medical and cost benefits. For the many scientists working to find a cognition activator with strong effects, the risk lies in the possibility that senile cognitive decline may not be treatable.

Dementia is a clinical syndrome involving reduced intellectual functioning with impairments in memory, language, visuospatial skills, and cognition including deficits in mathematics, abstraction, and judgement (Cummings, 1980). Currently, several forms of dementias such as multiinfarct dementia (MID), extrapyramidal disorders (EPS), depression, etc. have been treated (Cummings, 1985), but others cannot be treated, most notably primary

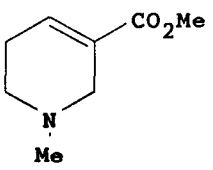
degenerative dementia (PDD; also called senile dementia, senile dementia of the Alzheimer type, Alzheimer's disease, organic brain syndrome). The original diagnosis of PDD was made in 1907 by Alois Alzheimer and the relationship between normal aging of the brain and PDD remains unresolved. PDD was considered a medical curiosity for many years. However, the magnitude of its occurrence, especially in the elderly, has only been recently appreciated. The etiology and pathogenesis of PDD is presently unclear, however, a number of factors have been hypothesized to be involved (Table I; Wurtman, 1985).

Table I. Possible Causes of PDD

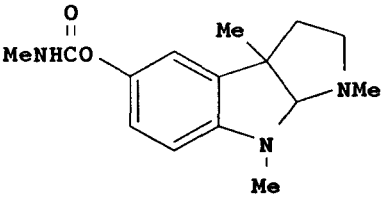
- genetic factors
- abnormal protein models
- infectious agents
- toxins
- blood flow disorders
- cholinergic hypothesis
- multiple factors

Within the last few decades, the focus of PDD research has shifted to biochemical and neurochemical approaches, with the hope of identifying agents that improve the behavioral endpoints of learning and memory by a defined mechanism of action. Present strategies include cholinergic agents (Table II), analogues of ACTH (adrenocorticotropin), vasopressin and somatostatin (Table III), serotonin agents, and adrenergic agents (Table IV).

Table II. Cholinergic Agents

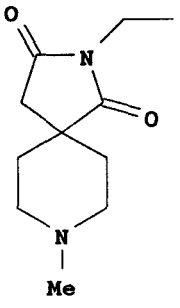


arecoline



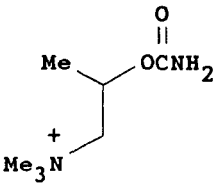
physostigmine

Flood, 1985; Mutschler, 1984; Mohs, 1985; Christie, 1981; Brinkman, 1983; Jotkowitz, 1983; Davis, 1982; Beller, 1985



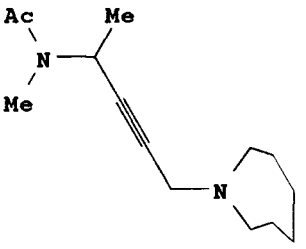
RS-86

Wettstein, 1984



bethanecol

Harbaugh, 1984



BM-5

Nordstrom, 19

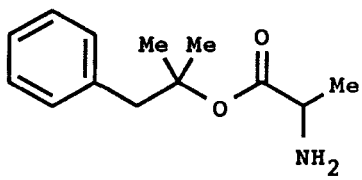
At present, the most widely accepted biochemical hypothesis involves the cholinergic system. PDD patients contain specific biochemical and histopathological changes in the cholinergic system. These observations suggest that new approaches are possible to develop animal models for senile cognitive decline.

Since the average age of the population is on the increase, the frequency of Alzheimer's disease is increasing rapidly, thereby requiring urgent attention (Goodnick, 1984).

Table III. Analogues of ACTH, vasopressin, and somatostatin

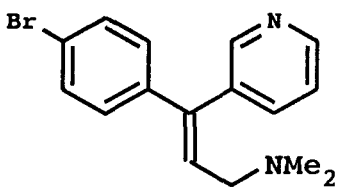
Met(S=O)-Gln-His-Phe-Lys-Phe **ORG 2766**	Nicholson, 1984 Jolkkonen, 1985 Nyakas, 1985
S-$(CH_2)_2$C(=O)-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-GlyNH_2 **DDAVP**	Beckwith, 1984 Stein, 1984
HCys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-OH **DGAVP**	Peabody, 1985 Jennekens-Schinkel, 1985
(N-Me)Ala-Tyr-D-Trp-Lys-Val-Phe **L 363,586**	Cutler, 1985

Table IV. Serotonin and adrenergic agents



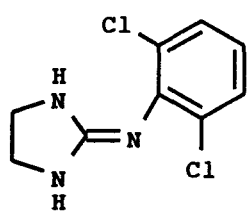
alaproclate

Dehlin, 1985 ; Bergman, 1983



zimelidine

Cutler et al., 1985



clonidine

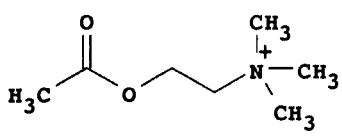
Arnsten, 1985

B. Acetylcholine and Cholinergic Receptors

1. *Acetylcholine (ACh)*

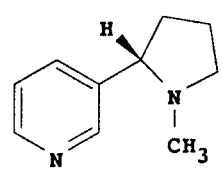
1.1. *Structure*

The cholinergic neuronal system can be found in the central nervous system (CNS; especially in the cortex and caudate nucleus), in the autonomic nervous system, and in the skeletomotor system. Acetylcholine (2-(acetoxy)-N,N,N-trimethylethanaminium ion; ACh) is the major neurotransmitter in the ganglia, the neuromuscular junction, and the postganglionic synapses of the cholinergic (parasympathetic) nervous system. Like most neuronal systems, cholinergic receptors show duality, and are distinguished as nicotinic and muscarinic receptors, which differ in many respects. Whereas acetylcholine (**1**) binds to both types of receptors, the plant alkaloids nicotine (**2**) and muscarine (**3**) trigger a response either from the nicotinic or muscarinic cholinergic receptors, respectively.



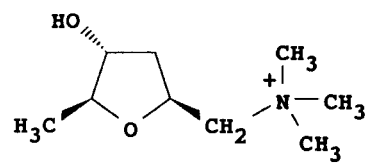
Acetylcholine

1



Nicotine

2



cis-(+)-Muscarine

3

1.2. *Conformation of ACh*

Owing to free rotation about the N-CH_2-CH_2-O side chain of acetylcholine, conformational isomerism must be considered an important determinant in assessing the interactions with different receptors, such as the nicotinic, the muscarinic, and the acetylcholinesterase (AChE; the enzyme responsible for maintaining the proper titer of ACh) receptor surface.

Much interest has been focused on the relationship of the torsion angle of the N-C-C-O ester fragment (τ_2) to the bioactivity (Fig. 3). The role of the torsion angle to muscarinic activity has been thoroughly examined (Portoghese, 1970; Pullman, 1972; Beveridge, 1973; Bergmann, 1974; Casy, 1975; Snyder, 1985). Among the many possible conformations, two predominant forms, the *gauche* and *anti*, should be considered.

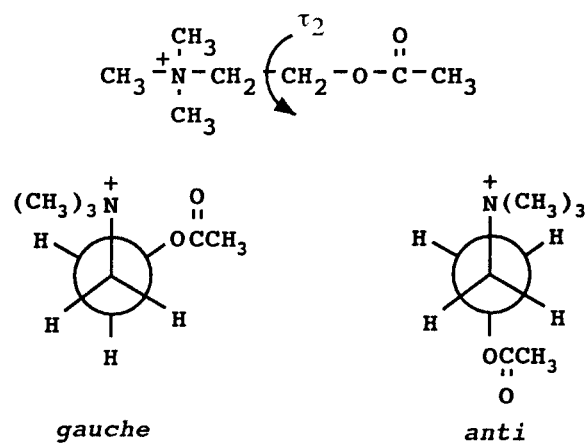


Figure 3. Structure and major conformations of acetylcholine

X-ray and NMR studies (Jagner, 1977; Partington, 1972; Casy, 1975) show that the N-C-C-O grouping of acetylcholine exists predominantly in the *gauche* conformation (Fig. 3). More detailed studies on the conformation of ACh and its relationship to biological activity will be discussed in Sect C.2.4.

1.3. *Metabolism, Release, and Storage of ACh*

Investigations of ACh metabolism have been studied using radioimmunoassay (Spector et al., 1978) and pyrolytic gas chromatography. These techniques can detect ACh at the femtomolar (10^{-15}M) level sensitivities.

Acetylcholine is synthesized by the following reaction (Eq. 1):

$$\text{Choline} + \text{Acetyl-CoA} \xrightarrow{\text{ChAT}} \text{Acetylcholine} + \text{CoA-SH} \qquad (1)$$

Here, choline is acetylated by acetyl-CoA as mediated by the enzyme, choline acetyltransferase (ChAT). Acetyl-coenzyme A (CoA) is ubiquitous and choline is obtained either from phosphatidyl choline (lecithin) or free choline. Some choline is obtained via recycling surplus ACh hydrolyzed by acetylcholinesterase (AChE) (Fig. 4). There also is a high-affinity transport system (Km = 1-5 M) for choline reuptake in the nerve endings, which can be inhibited by hemicholinium (4). Unlike most other neurotransmitters, however, ACh itself is not taken up by active transport into synapses.

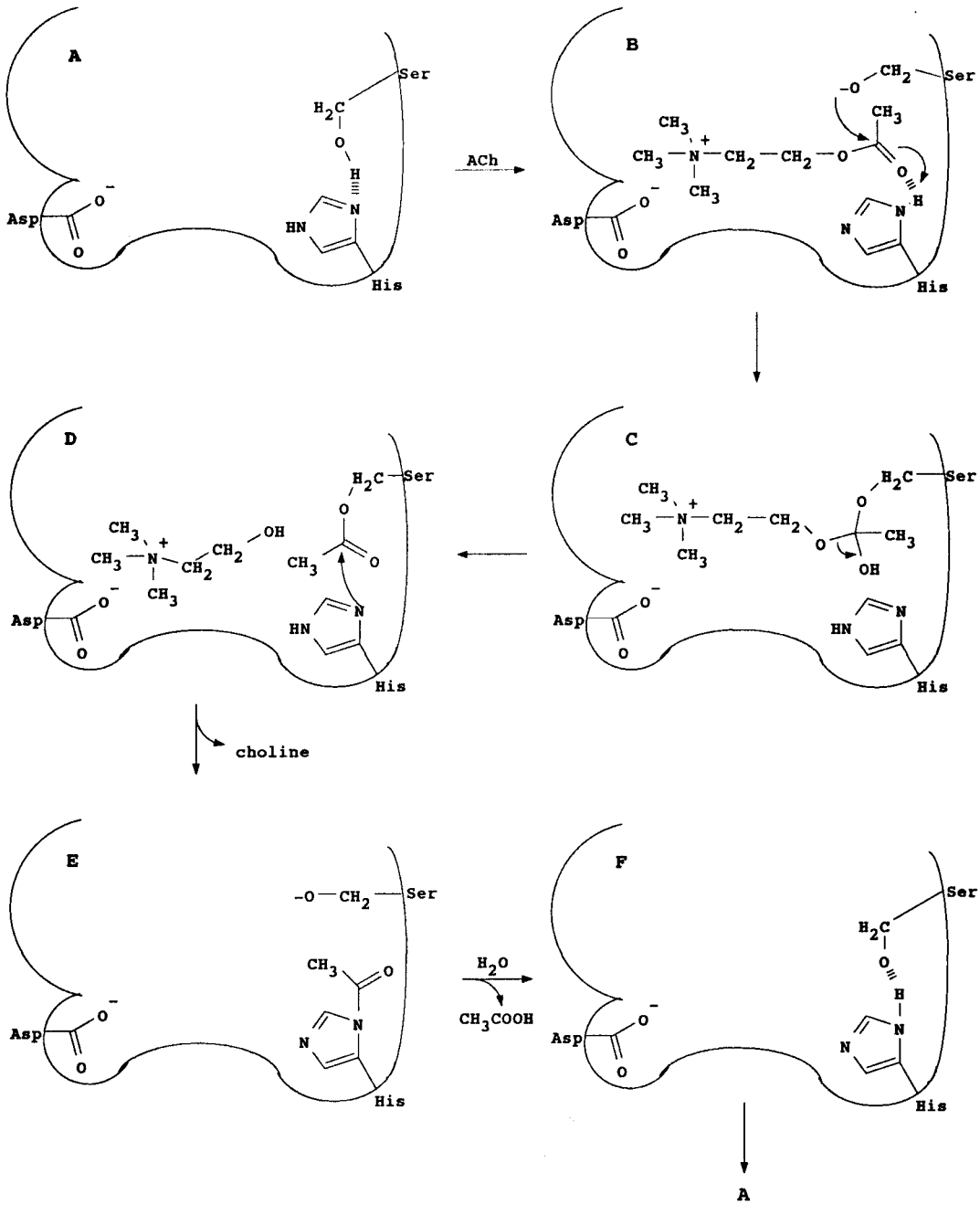
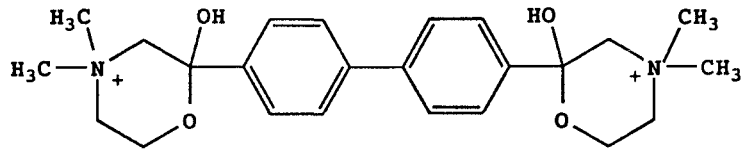


Figure 4. Mechanism of acetylcholine hydrolysis by acetylcholinesterase.



4

As ACh is synthesized, it is stored in the neuron or ganglion in at least three different states. Eighty-five percent of all ACh is stored in a "depot", and can be released by neuronal stimulation. It is always the newly synthesized neurotransmitter that is released preferentially. The "surplus" ACh can be released by K^+ depolarization only. Finally, there is "stationary" ACh, which cannot be released at all.

Whittaker (1986) has shown that synaptic vesicles are metabolically inhomogeneous, and those closer to the presynaptic membrane release ACh preferentially. Several alternative hypotheses on ACh release have been proposed. One proposal assumes a voltage-dependent Ca^+ reflux that opens a gate, allowing cytoplasmic ACh release for a timed period. A second proposes the containment of ACh in the smooth endoplasmic reticulum, in association with the presynaptic membrane. There is still much to be learned regarding the precise mechanism of ACh release (Tauc, 1982; Dunant and Israël, 1985).

2. *Acetylcholine Receptor*

2.1. *Anatomy*

The acetylcholine receptor (AChR) is one of the most thoroughly characterized components of the neuromuscular transduction process. Earlier reviews that summarized the structural and biochemical features of the AChR include those by Karlin (1980), Conti-Tronconi & Raftery (1982), Stroud (1983), and Popot & Changeux (1984). The AChR receptor translates the binding of the neurotransmitter, acetylcholine (ACh), into a rapid increase and subsequent decrease in the permeability of the endplate membrane to the passage of Na^+ cations. The availability of acetylcholine receptors from electric tissues (e.g., *Torpedo californica*, *Electrophorus electricus*, and *Torpedo marmorata*) was a fundamental key to molecular characterization. The subunit stoichiometry of the four identified polypeptides has been unequivocally established as $\alpha_2\beta\gamma\delta$, and the funnel shape of the molecule has been well characterized with respect to the position of the ion channel (McCarthy, 1986).

2.2. *Binding Sites*

The number and importance of different agonist binding sites on the AChR remains a matter of some controversy. A majority of studies indicate the presence of two, high-affinity ACh binding sites per AChR monomer in *Torpedo californica* localized on the α subunit (Conti-Tronconi & Raftery, 1982;

Popot & Changeux 1984). Several reports showed that these sites may not be initially equivalent (Weill, 1974; Damle, 1978; Weiland, 1979). Two classes of α subunits that differ in the extent of glycosylation have been observed in *T. californica* AChR (Conti-Tronconi *et al.*, 1984). These differences may contribute to the nonequivalence of the high affinity agonist binding sites. Although these sites may not be identical in structure, both are involved in channel opening.

The location of agonist binding sites remains a topic of great interest. The bulky, quaternary amine head group of the agonist probably binds to a sterically-restricted, anionic region on the receptor, while hydrogen bond formation between the carbonyl of ACh and the receptor AChR lend additional binding energy (Spivak and Albuquerque, 1982).

C. Stereochemistry and Conformation of Drug-receptor Interaction

1. *General Aspects*

One of the principle goals in pharmacology and medicinal chemistry is to adequately describe the molecular dynamics of the interactions between a drug and its receptor(s). These interactions exist in a system in which two components are essential: (1) the precise structure of the drug, and (2) the structure of the binding site(s) of the receptor macromolecule with which the drug interacts. Since all receptors are topologically defined, any description of a pharmacophore or drug must be three dimensional. As long as detailed stereochemical information on receptors is lacking, a logical step is to look at the stereochemical anatomy of the drug molecules interacting with a given receptor. In this manner, certain 3-D complementary features of the receptor can be gleaned. A great deal of effort has, therefore, been devoted to studying the relationship between stereochemistry and the active conformation of flexible drug molecules that trigger the response.

One approach to the study of conformational selectivity at drug receptors has been the evaluation of preferred (lowest energy) conformation of the isolated molecule (quantum chemical, and empirical or force field calculations), of the molecule in the crystal state (X-ray crystallography), and of the molecule in solution (NMR spectroscopy). But attempts to deduce the receptor-active conformation of flexible drug from these studies alone are meaningless. Whether the methodology is theoretical, crystallographic, or spectroscopic, the

studied environment (vacuum, crystal, or solution) generally neglects possible higher energy and tight binding conformations that can occur in the flexible drug molecule through interaction with the receptor. Such possible conformations have generally been ignored, focusing on the energetically most stable conformer (Portoghese, 1970; Reed 1981).

Ariëns (1984) outlined and elucidated the significance of stereochemistry in therapeutic action and the role of enantioselectivity in 'hybrid' drugs (Ariëns 1986). Stereoisomerism and drug action (Lehmann 1986) has also been reviewed. Even earlier, the significance of stereoselectivity and affinity in molecular pharmacology was perceived in detail by Lehmann et al. (1976). A number of reports on substrate dependent enantioselective metabolism of chiral CNS agents, autonomic and cardiovascular agents, and miscellaneous drugs and xenobiotics were also reviewed (Low et al., 1978).

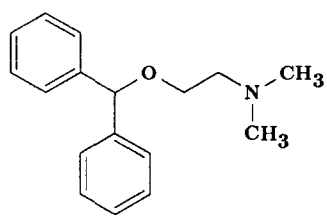
2. *Rigid or Semi-rigid Drug Analogues*

2.1. *Methods of Reducing Conformational Flexibility*

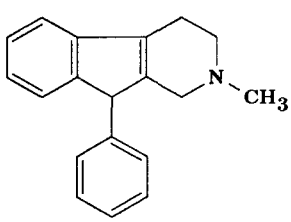
Four different techniques of controlling the geometry of a flexible drug molecule, and of restricting rotations within the molecule may be used.

Making use of steric factors; The freedom to rotate about a σ-bond may be limited if the atoms forming a bond have large groups attached to them. In extreme cases, as with some di-*ortho*-substituted biphenyls, the rotation is

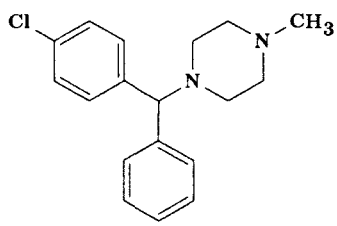
sterically restricted to give two distinct optical enantiomers. The use of this approach was documented in a series of diphenhydramines (Harms et al., 1960). The authors investigated whether the ortho substituents weaken antihistaminic activity by interfering with the possibility for the flexible side chain to take the curled up position, which in the rigid structures mentioned is associated with high activity (e.g., orphenadrine and neo-benodine). These studies were conducted in view of the fact that several very active antihistaminics possess completely rigid or semi-rigid structures, the side chain either being fixed to one of the rings (phenindamine), or able to rotate around one axis only (chlorocyclizine).



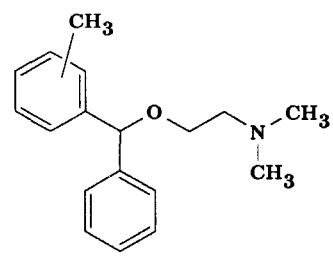
Diphenhydramine



Phenindamine



Chlorocyclizine



ortho-; Orphenadrine
para- ; Neo-benodine

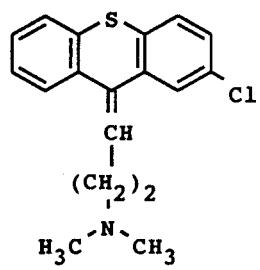
Making use of bioisosterism; Bioisosters are substituents or groups that have chemical or physical similarities that can produce broadly similar biological properties (Thornber, 1979). There are several literature studies in which the principles of bioisosterism were used to deduce stereostructure-activity relationships. A selected few are listed in Table V.

Table V. Making use of bioisosterism

$(CH_3)_3N^+CH_2CH_2$-B-R Choline isologs $(CH_3)_3N^+CH_2CH_2CH_2$-B-R Homocholine isologs where, B = O, S, Se R = -H, $-CH_3$, $-COCH_3$	Webb et al., 1966
$(CH_3)_3N^+CH_2CH_2C(O)CH_3$ I^-	Jagner and Jensen, 1977
A || $CH_3CBCH_2CH_2N^+(CH_3)_3$ where, A = O, S B = O, S, Se	Makriyannis et al., 1972 Chidichimo and Russo, 1977 Pullman and Courriere, 1972 Aldrich, 1975
A || $C_6H_5CBCH_2CH_2NR_2$ where, A = O, S, Se B = O, S, Se, NH R = $-CH_3$, $-CH_2CH_3$	

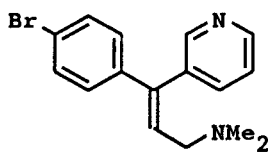
Making use of multiple bonds; The relative positions of atoms attached directly to multiple bonds are fixed. In the case of double bonds, *cis* and *trans* isomers results (Table VI). The fixed geometry of the alkene differs greatly from the conformational freedom inherent in the corresponding alkane structure.

Table VI. Making use of multiple bonds



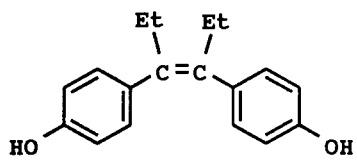
2-chloro-9-(ω-dimethylaminopropyliden)thioxanthene

Dunitz, 1964



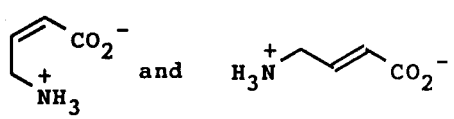
zimelidine

Ross, 1977
Coppen et al., 1979
Brown et al., 1980



cis- and *trans*- Stilbestrol

Solmssen, 1945



cis- and *trans*- 4-Aminocrotonic acid

Johnston et al., 1975
Allan et al., 1980

Making use of cyclization; The conformational mobility of flexible drugs can be further reduced by incorporating various parts of the molecule into a ring structure. It is a simple matter to find rigid or semi-rigid skeletons into which the essential structure of drugs (the appropriate functional groups for potential activity) may be incorporated, and a lot of attention has been focused on the receptors of acetylcholine (Sections, B.2.2. and C.2.4.).

2.2. *Advantages of Controlling the Geometry of Flexible Drugs*

The major advantages of controlling conformational flexibility are: (1) the configuration of the active pharmacophoric conformation can be determined; (2) the key functional groups are rigidly held in one position, and a semi-rigid structure constrains these groups to certain values. Within a set of rigid or semi-rigid analogues, it is assumed that only those which fit the receptor will be active. In certain cases, stereochemistry is exclusively responsible for the differences in the degree of biological activity between the flexible parent drug and the constrained derivatives. From a comparison of the stereochemistry of the active analogues with possible conformations of the original drug, we can obtain information about which of the conformations of the parent substance reacts with the receptor and about the barriers to conformational alteration during drug-receptor interaction.

2.3. *Disadvantages and Limitations of Controlling the Geometry of Flexible Drugs*

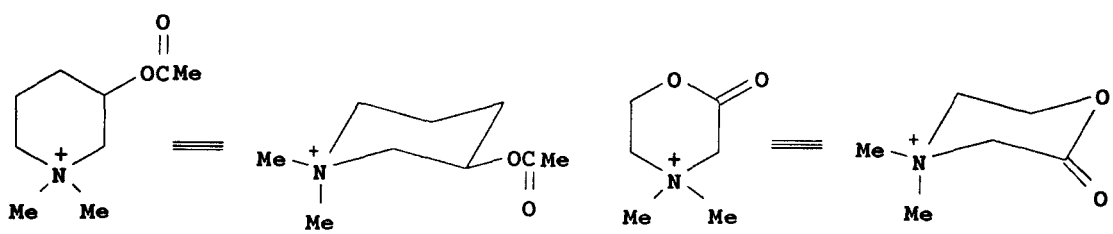
The use of conformationally constrained analogues of flexible drug molecules to investigate the participation of conformational isomerism in drug activity appears to be a feasible approach, although it is not without pitfalls. It is very difficult, if not impossible, to control the geometry of a flexible drug without also changing some other physico-chemical properties of the prototype drug. To produce a conformationally constrained analogue, new atoms and/or bonds must be added, and this may impart different chemical and physical properties, which must be taken into consideration when interpreting subsequent biological data. There is another problem in relating conformational isomerism with biological activity using rigid or semi-rigid analogues. Rigidity may deny a molecule the opportunity of undergoing a necessary conformational change during its interaction with the receptor. Therefore, analogues representing a highly rigid conformer could furnish misleading results when some degree of flexibility is needed for ideal interaction with the receptor. This feature is best described as mutual adaptability (Bergmann, 1974).

Beers and Reich (1970) and Burgen (1975) proposed that molecular flexibility finds expression in the kinetic parameters of drug-receptor interaction. Lass et al. (1979), studying the time course of the action of muscarinic antagonists in carp atria, found that the dissociation of rigid

antagonists was very much prolonged as compared to flexible drugs of the same affinity. By way of contrast, Wassermann et al. (1979) reported that structure flexibility plays no role in the mechanism of action of acetylcholine at its receptor. In Wasserman's study, highly constrained depolarizing ligands were used.

2.4. *Studies with Cyclic Analogues of ACh*

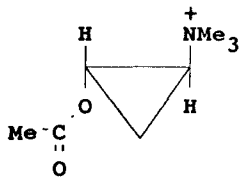
Schueler (1956) first drew attention to the possibility that muscarinic and nicotinic effects due to ACh are mediated by different conformational isomers of the molecule. He examined the N,N-dimethyl-3-acetoxypiperidine (**5**) and the morpholine derivative (**6**) as models of antiplanar and synclinal N/O conformations of ACh, respectively. Both were feebly active in muscarinic and nicotinic assays, with (**5**) showing greater potency.



5 6

Cannon's group chose the cyclopropane ring as the smallest system capable of conferring conformational and configurational rigidity on an ACh analogue (Armstrong, 1968; Chiou, 1969), and succeeded in obtaining an

isomer that had a high muscarinic potency.



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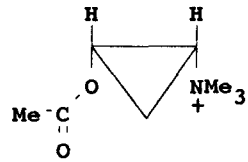

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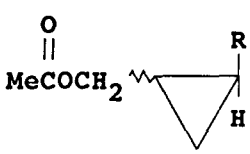
(+)-*Trans*-2-acetoxycyclopropyltrimethyl ammonium iodide (ACTM) (**7**) equalled or surpassed ACh in two biological test systems. Interestingly, the enantiomer (-)-*trans*-ACTM was several hundred times weaker than the (+)-form while the racemic cis isomer (**8**) was virtually inactive. Cyclopropyl and cyclobutyl derivatives (**9** - **11**) have also been examined (Cannon, 1972, 1973) as analogues of acetyl γ-homocholine or 4-acetoxybutyltrimethyl ammonium, but provided no useful data relevant to the active conformation of ACh.



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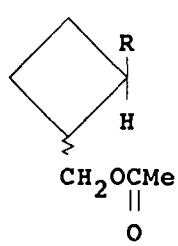

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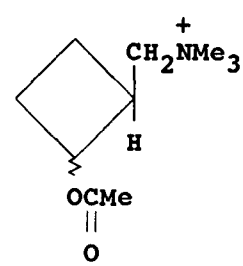
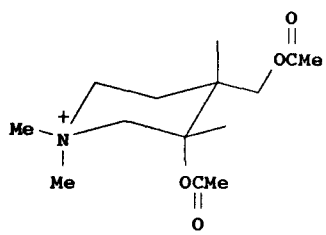


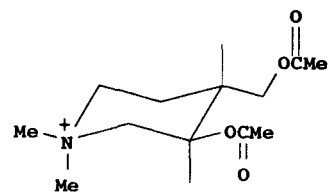
11

R = $\overset{+}{N}Me_3$ or $CH_2\overset{+}{N}Me_3$

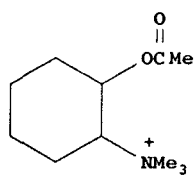
Some cyclohexyl analogues of ACh are illustrated in Table VII. More cyclic analogues of ACh will be introduced in the following section as cyclic imonium ions.

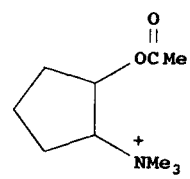
Table VII. Cyclohexyl derivatives of acetylcholine



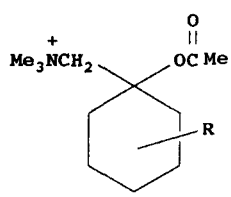


Lewis et al., 1973



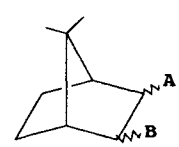


Baldridge, 1955; Friess, 1956



R = H, 2-Me, 3-Me, and 4-Me
(where R = Me, cis and trans isomers separated)

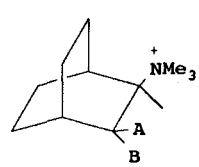
Biggs, 1972



a) A = OC(O)Me, B = $\overset{+}{N}Me_3$

b) A = $\overset{+}{N}Me_3$, B = OC(O)Me

Chittenden, 1970; Ahmad, 1971; Cooper, 1971; Beckett, 1971



a) A = OC(O)Me, B = H

b) A = H, B = OC(O)Me

Nelson, 1971; Beckett, 1972

D. Aziridinium Analogues

1. *Aziridinium (Ethyleneimonium) Cation*

The aziridinium cation was recognized as the key intermediate in chemical reactions of nitrogen mustards, β-haloethyl-*tert*-amines and as their pharmacologically active species in alkylating functional groups of compounds of biological importance, particularly, in adrenergic blocking and in antiadrenaline and antihistamine activity (Gilman and Philips, 1946; Golumbic *et al.*, 1946).

The majority of the nitrogen mustards are *bis*(β-chloroethyl)amines, the third valence of nitrogen being occupied by one of a variety of alkyl groups. The rate of cyclization and the activity of the aziridinium cation is influenced by certain substituent groups on the molecule. A large number of nitrogen mustards of different physicochemical and pharmacological properties have been prepared. Thus, the scope of future investigations on the relationships between chemical constitution and pharmacological action of the nitrogen mustard is broad.

It is useful to first describe the fundamental properties and formation of the aziridinium species. In pure aqueous solutions at physiological pH the aziridinium cation reacts with H_2O (Fig.5). However, if other substances, namely nucleophiles are present, they can react competitively. More detail regarding this reaction will be discussed in Chapter III.

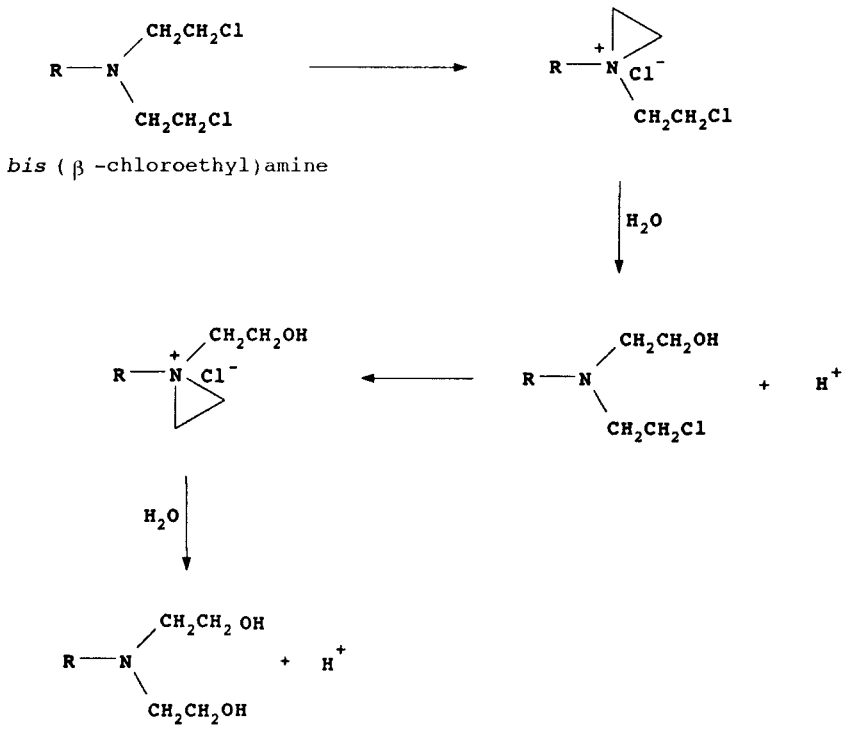


Figure 5. Reaction of aziridinium cation with water.

2. *Acetylcholine Mustard Analogues*

The first synthesis of acetylcholine mustard (AChM) can be attributed to Hanby and Rydon (1947), and its toxicology and other nitrogen mustard analogues of choline can be credited to Anslow et al.(1947). Jackson and Hirst (1972) modified the synthesis procedure to obtain a higher yield, and showed clearly that the biological activity of AChM was due to formation of the aziridinium ion in a polar solvent. Comprehensive investigations of a variety of aziridinium analogues of choline and acetylcholine were reported by Clement and Colhoun (1974), Rylett and Colhoun (1977; 1979; 1980a,b; 1985).

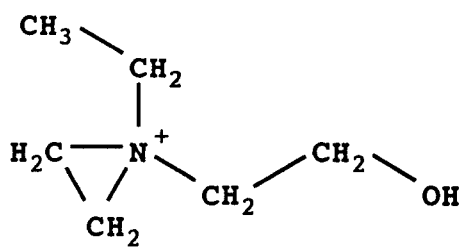
3. *Ethylcholine Mustard Aziridinium; AF64A*

Much information has been provided over recent years showing the importance of specific neurotransmitters to study the etiology of various neuropsychiatric diseases. To understand neurotoxins in a particular neurotransmitter system *in vivo*, a number of attempts have been explored utilizing compatible neurotoxins. Such agents would be extremely valuable on two levels. First, they could be used to mimic specific neurotransmitter deficits shown to occur in certain disease states (e.g., dopamine in Parkinson's disease; acetylcholine in Alzheimer's disease), and thus attempt to reproduce the biochemical abnormality. Second, neuron-specific neurotoxic agents could serve as chemical probes in the study of basic mechanisms pertaining to the synthesis and metabolism of the affected neurotransmitter system, *in vivo* (Hanin *et al.*, 1987). This Hanin group's suggestion implies the requirement of synthesis of a compatible, neuronspecific neurontoxin.

Under normal circumstances, the rate of neuronal metabolism and turnover of ACh is extremely rapid, much faster than any of the other known neurotransmitters. Therefore, the cholinergic system generally is highly resistant to imitate. For this reason, studies of events at the cholinergic nerve terminal have been difficult, particularly when conducted in the intact mammalian system. Available pharmacological agents that have been able to perturb the cholinergic system *in vivo*, whether as agonists (e.g., cholinesterase inhibitors, pilocarpine, arecoline, oxotremorine, etc.) or antagonists (e.g.,

atropinic agents, hemicholinium-3, etc.), usually have a limited period of action (hours or fractions thereof). Such agents have been useful as tools in evaluating acute responses and charges at the cholinergic nerve terminal. However, considering the study on the consequence of long-term cholinergic perturbation *in vivo*, these agents are of less use. A long-acting neurotoxin would be extremely valuable for this purpose. Furthermore, it could simulate the condition believed to exist in the case of Alzheimer's disease, i.e., a long-term, persistent cholinergic hypofunction in the central nerve system (Hanin et al., 1987)

Fisher and Hanin (1980) introduced AF64A (Ethylcholine Mustard Aziridinium, ECMA, **12**) as a cholinergic neuronspecific neurotoxin and Hanin and colleagues later demonstrated that this compound may have selective cholinergic actions in the central nervous system of mice and rats (Fisher, 1983; Hanin, 1983; Mantione, 1981, 1983, 1984). Treatment with ECMA and ACh mustard analogues *in vivo* simulate the neurochemical conditions that appear to cause a number of neuropsychiatric diseases including Alzheimer's disease, have been reported (Curti, 1984; Sandberg, 1985; Mistry, 1986; Pittel, 1987; Laganiere, 1990).



12

4. *Mechanism of Action of AF64A*

Unlike the noncovalent ACh-AChR interaction, the highly strained three-membered ring structure of AF64A causes a unique interaction with the receptor, by forming a new covalent bond between a carbon of its ring and nucleophilic functional group in the anionic locus. The initial attraction to the receptor is probably governed by electrostatic force interaction between charged species. The possible mechanism of blocking the binding site by the AF64A is illustrated in Fig.6.

Whether the new covalent bond stays, or eventually breaks by other factors, the new covalent bond has a much longer life time compared to the native neurotransmitter, and its receptor binding state.

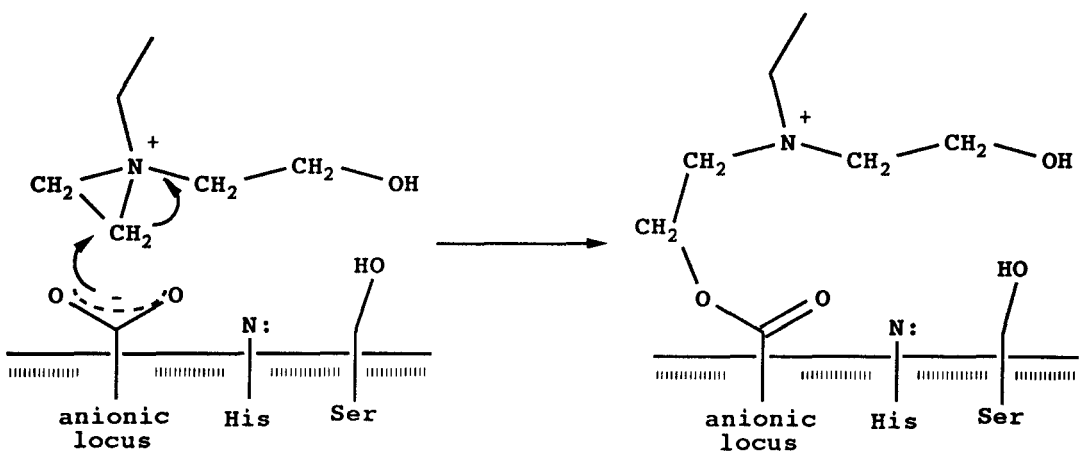


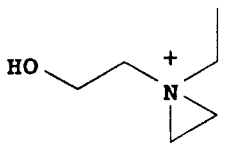
Figure 6. Possible blocking mechanism of the AF64A in the receptor binding site.

CHAPTER II

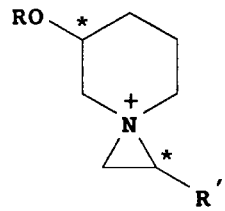
STATEMENT OF GOALS

Although AF64A received a great deal of attention as a selective cholinergic neurotoxin, there are still several aspects of the approach to be examined and developed. Therefore, we would like to support the needed information through the preparation of chiral derivatives with the hope that these supportive studies could be used in furthering the development of AF64A-based drugs and prodrugs.

First, as mentioned earlier in the introduction, the importance of stereochemistry in a biological molecule can be discussed as related to this neuron-specific neurotoxin. In terms of structure, AF64A simply blocks the binding site of its receptor by formation of a new covalent bonding between a carbon of the aziridinium mustard ring and a nucleophilic moiety at the binding site. However, AF64A does not give any configurational or stereochemical information of the receptor binding site raising the question of which configurational isomer shows higher affinity/reactivity toward its receptor. Therefore, we wish to undertake the synthesis of heterospirocyclic mustard analogues of AF64A by imposing chiral center(s) on those analogues, in the hope of enforcing a distinct stereochemical environment upon the neurotoxins.

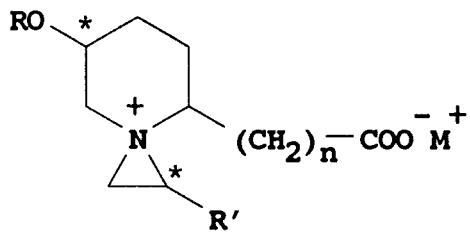


AF64A



R = H or OAc
R' = H or Me
* : chiral centers

Second, as a charged small molecule, AF64A may have problems penetrating through the blood-brain barrier, and would be easily solvated and decomposed in the biological system before the molecule reaches the binding site. Hence, modifying our synthetic pathways, we can expand the size of analogues by attachment of a long side chain to render them recognized more easily. The additional hydrocarbon chain would provide enhanced lipophilicity that could possibly make the molecules penetrate the blood-brain barrier better.



Finally, as an organic synthetic chemist, the author had a great desire to isolate spirocyclic aziridinium ion analogues rather than generating the reactive species *in situ*. The formation of aziridinium ion in buffer solution (*in situ*) gave somewhat inconsistent results from some research groups (see Chapter III) due to the instability of the aziridinium ion during the incubation

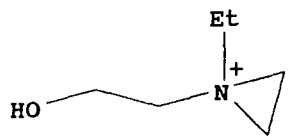
period. Obtaining purified salts of aziridinium analogues in itself would have great advantages of knowing the exact molar concentrations of these putative neurotoxins in biological assay (*in vitro* and *in vivo*). Although no stable, spirocyclic aziridinium analogue of AF64A has ever been prepared, the long term benefits of the goal are deemed highly significant.

CHAPTER III

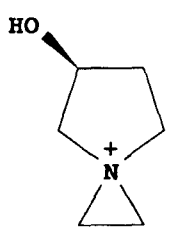
PROJECT DESIGN AND TARGET MOLECULE SELECTION

A. Cyclic Analogues of AF64A

In order to focus the design and the construction of our target molecules, we first wished to study the structure of AF64A and collect detailed information related to the topography of this neurotoxin and related compounds. This approach would be an ideal starting point with the study extended to possible bi- or spiro-cyclic analogues. In case of bicyclic analogues, azabicyclo[3.1.0]hexanes (**15** and **16**) and azabicyclo[4.1.0]heptanes (**19** and **20**) are proposed (Fig. 7). On the other hand, as appropriate spirocyclic analogues, 5-hydroxy-3-azoniaspiro[2.4]heptanes (**13** and **14**) and 5-hydroxy-3-azoniaspiro[2.5]octanes (**17** and **18**) are proposed. From a molecular structural point of view, all of these analogues and AF64A show a common feature; namely, two carbons are located in between the nitrogen and oxygen atoms. However, the asymmetrical cyclic structure of these derivatives of AF64A furnishes each analogue with chirality.



12: AF64A



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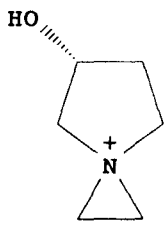
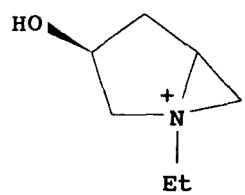


14

spiropyrrolidines



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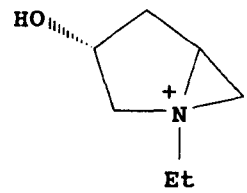
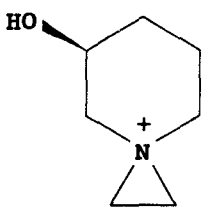


16

[3.1.0]
azabicyclohexanes



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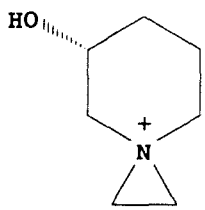

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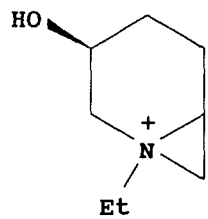
spiropiperidines



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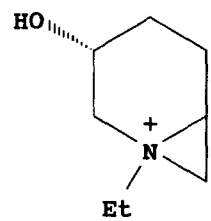

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[4.1.0]
azabicycloheptanes

Figure 7. Proposed analogues of AF64A.

The proposed AF64A derivatives represent conformationally and configurationally set analogues. It is possible to estimate via computer simulations each minimized conformation, dihedral angle, and intramolecular atomic distance between the two heteroatoms, nitrogen and oxygen. We conducted molecular mechanics calculations on choline, AF64A, and three derivatives. The results are shown in Table VIII (PCMODEL, 1988 Version).

Table VIII. Molecular mechanics calculations

Molecule	Intramolecular N-O distance (Å)	Dihedral angle (°)
Choline (*gauche* rotomer)	3.01	57.43
Choline (*anti* rotomer)	3.79	178.15
AF64A (*gauche* rotomer)	3.00	63.39
AF64A (*anti* rotomer)	3.74	174.62
(S)-3-Hydroxyspiropiperidine mustard (**17**)	2.89	65.09
(R)-3-Hydroxyspiropiperidine mustard (**18**)	3.71	175.23
(S)-3-Hydroxy-[4.1.0]-aza-*trans*-bicycloheptane ($\mathbf{19}_{trans}$)	2.69	63.74
(R)-3-hydroxy-[4.1.0]-aza-*trans*-bicycloheptane ($\mathbf{20}_{trans}$)	3.62	173.41
(S)-3-Hydroxy-[4.1.0]-aza-*cis*-bicycloheptane ($\mathbf{19}_{cis}$)	2.95	66.47
(R)-3-Hydroxy-[4.1.0]-aza-*cis*-bicycloheptane ($\mathbf{20}_{cis}$)	3.72	170.50

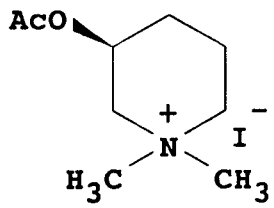
The *gauche* rotomers of choline and AF64A show identical intramolecular atomic distance between oxygen and nitrogen, and the *anti* rotomers show only a 0.05Å difference in intraatomic distance. (S)-3-Hydroxyspiropiperidine mustard (**17**) can be considered as a configurationally locked analogue of a *gauche* rotomer of choline or AF64A that deviates by only 0.11Å (in intraatomic distance from AF64A). This difference is much shorter than even the hydrogen atomic radius (0.53Å). The (R)-isomer (**18**) also shows a well matched intraatomic distance when compared with the *anti* rotomers of choline and AF64A. The dihedral angle value of this isomer is even closer to the value of choline than that of AF64A. The bicyclic [4.1.0] mustard derivatives were analyzed as *cis* and *trans* isomers relative to the stereochemistry of the ring junction. The *cis* isomers of (**19**) and (**20**) show similar intraatomic distances to (**17**) and (**18**), respectively. However, the dihedral angles of these derivatives show somewhat higher deviations.

B. Prior Studies on Piperidine-based ACh Analogues

As mentioned in the introduction, conformationally rigid or semi-rigid analogues of flexible drugs can offer a powerful aid toward establishing the conformational and configurational requirements of pharmacological receptors. This, in turn, may lead to enhanced selectivity of current drugs, and the design of new active agents. Before we embark on a synthesis of the preliminary target molecules, we first should be aware of several studies performed earlier that could help us to design and construct our heterocyclic spiropiperidine mustard derivatives. In the following sections, we will describe how these studies relate to our proposed derivatives.

1. *3-Acetoxypiperidine Derivatives*

With interest in study of the physiological neurotransmitter acetylcholine, Lambrecht and Mutschler (1979) investigated the muscarinic activities of piperidine derivatives as conformationally and configurationally fixed analogues of acetylcholine. First, they synthesized the tertiary and quaternary individual enantiomers of N-methyl-3-acetoxypiperidine, and N,N-dimethyl-3-acetoxypiperidiniums (**21-24**) and later tested these compounds for their muscarinic activities (Lambrecht and Mutschler, 1974a,b; Höltje, 1979; Lambrecht, 1976a,b; Lambrecht 1980).



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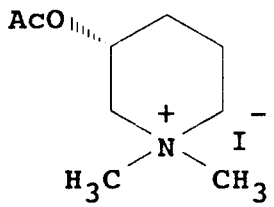

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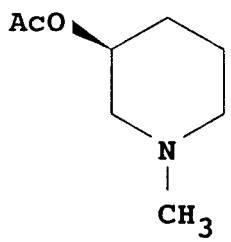

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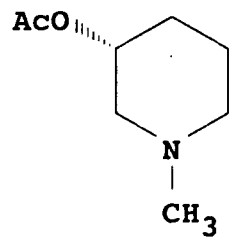

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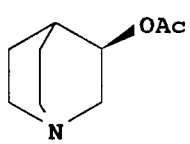
As shown in Table IX, all the semi-rigid analogues, based on the piperidine skeleton, were very weak muscarinic agonists. In this table, the pD_2 value is a measure of muscarinic potency, and the higher value shows higher potency. The low muscarinic potency of these compounds might result from structural and stereochemical restrictions. Lambrecht and Mutschler forwarded two possibilities for these weak activities; (1) too much energy is needed for the cyclic analogues to reach the required muscarinic-essential conformation during agonist-receptor interaction, and (2) direct steric hindrance caused by the supporting structure of the compounds.

Table IX. Muscarinic activities of cyclic acetylcholine analogues in the piperidine series.

Compound	pD_2
Acetylcholine (**1**)	7.51
(S)-N,N-Dimethyl-3-acetoxy-piperidinium iodide (**21**)	3.92
(R)-N,N-Dimethyl-3-acetoxy-piperidinium iodide (**22**)	3.40
(S)-N-Methyl-3-acetoxypiperidine (**23**)	3.68
(R)-N-Methyl-3-acetoxypiperidine (**24**)	2.99

These questions were also addressed, in part, by Mashkovsky (1963), who imposed further conformational restrictions on these piperidine compounds by an additional bridging ethylene resulting in 3-acetoxyquinuclidines (**25**-**28**).



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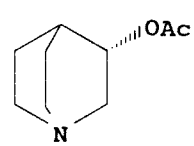

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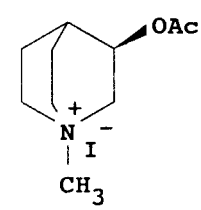

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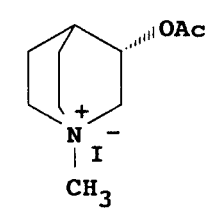

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The formal representation of 3-acetoxyquinuclidine's ring system consists of three boat like structures of piperidine, where each boat shares the nitrogen and three other carbon atoms with the other's boats (Fig. 8).

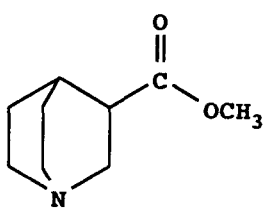
Figure 8. (S)-Boat conformation of N-methyl-3-acetoxypiperidine (**23**) and the structure of the (S)-enantiomers of 3-acetoxyquinuclidines; R = H (**25**), CH_3 (**27**).

As shown in Table X, the tertiary quinuclidine esters (**25** and **26**) have considerable muscarinic potency, and the more potent (S)-enantiomer may be used as a model for discussing fit to the receptor. First, the high activity of the tertiary compound indicates that the parts of the quinuclidine ring, which are not present in acetylcholine, only slightly disturb the approach to, or the reaction with, the muscarinic receptor. Second, the muscarinic potency of the corresponding quaternary compounds (**27** and **28**) is much weaker, probably caused by steric hindrance due to the additional equatorial N-methyl group of the compounds.

Table X. Muscarinic activities of cyclic acetylcholine analogues in the quinuclidine series.

Compound	pD_2
Acetylcholine (**1**)	7.51
(S)-3-Acetoxyquinuclidine (**25**)	6.10
(R)-3-Acetoxyquinuclidine (**26**)	5.02
(S)-3-Acetoxyquinuclidine methiodide (**27**)	3.97
(R)-3-Acetoxyquinuclidine methiodide (**28**)	3.56

Mutschler's group (Gloge, 1966; Kummer, 1966; König, 1967; Christiansen, 1967) also investigated the corresponding inverse esters, namely methyl quinuclidine-3-carboxylate (**29**) and methyl N-methyl-piperidine-3-carboxylate (dihydroarecoline; **30**) and evaluated their muscarinic action.



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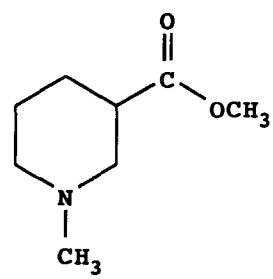

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These inverted esters showed very similar activities to their 3-acetoxy-derivatives. This means that the constitution of the ester side chain is not important for their affinity in this case. It is also unimportant whether the structure contains the ester of an amino acid with an alcohol or with the ester

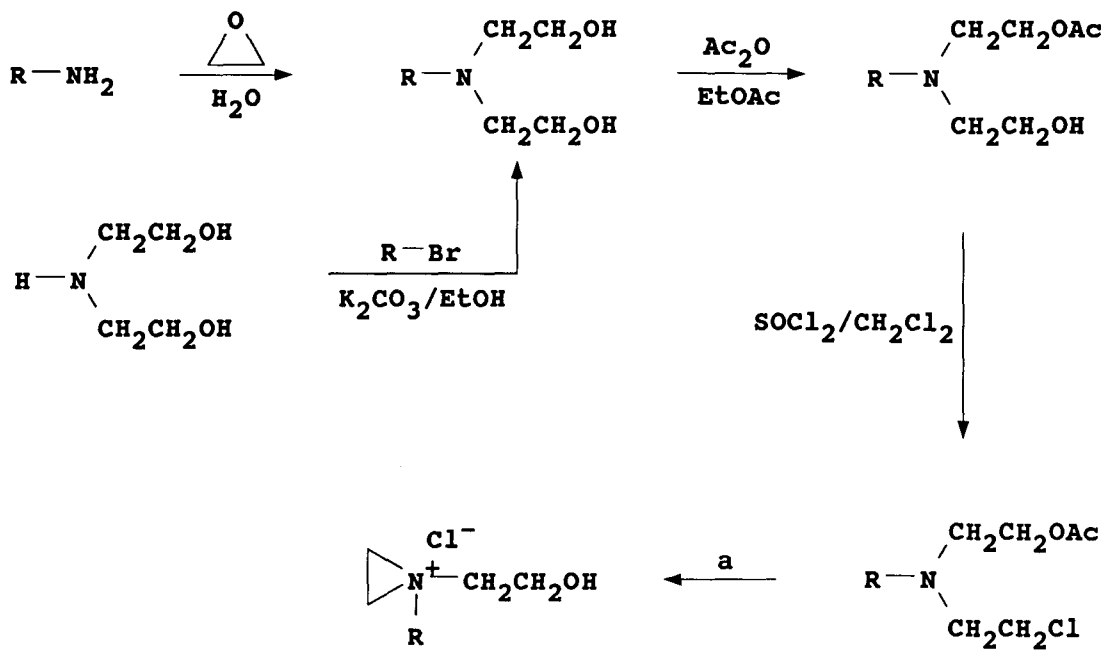
of an amino alcohol with a carboxylic acid. It is the rigid boat form of the quinuclidine ring which is the necessary characteristic for its activity.

Lambrecht and Mutschler's work suggested to us that our proposed spiropiperidine aziridinium analogues would show certain muscarinic activities. Also, we could opt to replace the hydroxyl group with an acetoxy group at the C_3 position. Considering the high reactivity of the aziridinium ring, replacing the hydroxyl group with acetoxy group may permit these analogues to survive longer until they reach at the acetylcholine receptor sites without going through the interaction with acetyl CoA and ChAT to induce the acetylcholine analogues.

2. *Studies on Aziridinium-based Molecules Related to AF64A*

After Fisher and Hanin (1980) proposed that nitrogen mustard analogues of choline could be the cholinotoxins of choice for the development of animal models of human neurologic and psychiatric disorders involving the cholinergic system (e.g., Alzheimer's disease), some of the more than 126 reports produced evidence for the cholinergic effect of AF64A in the CNS of rodents. A small number of investigators have found difficulties with use of AF64A, the most notable of which is nonspecific tissue damage at the site of injection, which could preclude the selectivity of its reaction. The general method of preparation of AF64A and its derivatives is shown in Scheme I (Mistry, 1986).

Scheme I. Synthesis of AF64A



a 1. $NaOH/H_2O$; pH 11.5 (30 min), 2. H_2O; pH 7.4
R = Et (AF64A), *n*-Pr, *i*-Pr, cyclopropyl, *n*-Bu, *i*-Bu

After the 2-[alkyl(2-hydroxyethyl)amino]ethyl acetates were obtained, cyclization to the desired aziridinium choline moieties was accomplished by alkaline hydrolysis of the acetylated compounds. This step can lead to discrepancies in dose and biological effect owing to incomplete conversion to aziridinium causing inconsistent results for some investigators. For example, formation of the nitrogen mustard required incubation of the β-chloroethyl precursor at high pH (11.5) for up to 30 minutes. The longer the time the cyclized compound is maintained at alkaline pH, the less aziridinium ion will

be recovered since the aziridinium ion itself is susceptible to hydrolysis. Rylett and Colhoun (1980) found that such prolonged exposure to high pH is not necessarily required for hydrolysis of the acetoxy bond, and a lower pH accomplishes the desired effect. In their report, maintaining the mustard compound at pH 10.5-11.0 for 10 minutes is adequate for complete generation of the choline moiety.

The transformation of mustard into aziridinium ion was detected by the iodine-thiosulphate titration method (Golumbic, 1946). The solvated aziridinium ion is unstable and decomposition, largely to the alcohol (Fig. 9, reactions **3** and **5**), is both temperature and time dependent. At ambient temperature and neutral pH, the rate of decomposition of acetylcholine mustard aziridinium ion is 5% in 90 minutes (Jackson and Hirst, 1972).

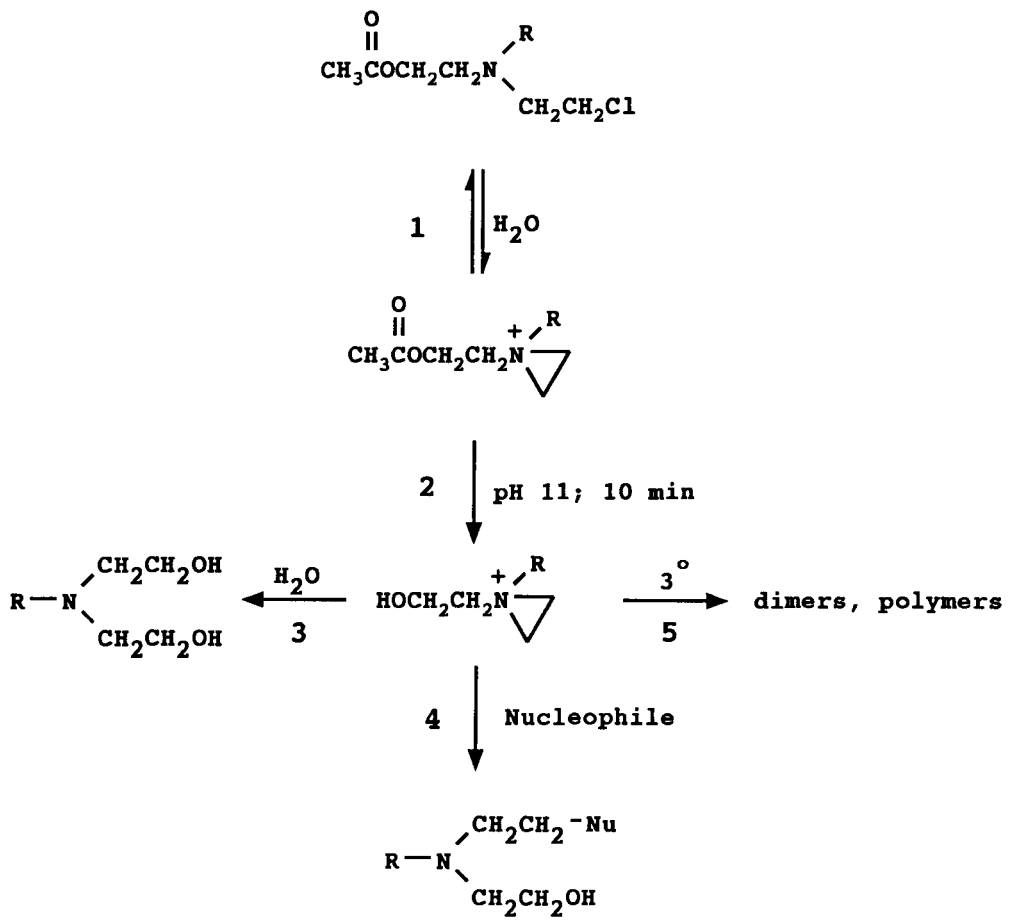


Figure 9. Structure and reaction scheme for nitrogen mustard analogues of choline.

As shown in Fig. 9, in aqueous solution, tertiary amines bearing the β-chloroethyl moiety undergo spontaneous intramolecular alkylation by reversible reaction **1** to yield the aziridinium ion and displaced Cl^- (Golumbic, 1946). At ambient temperature and physiological pH, approximately 70% of the aziridinium is formed when 10 mg / mL acetylcholine mustard is dissolved in water (Rylett, 1979).

The cyclized quaternary amine produced is vulnerable to a number of ring opening reactions with the net result of lowering the effective concentration of biologically active compound in solution. These reactions include recombination with the leaving group halide to yield the tertiary amine (reaction **1**), hydrolysis by reaction with water to yield the alcohol (reaction **3**), alkylation of nucleophilic groups in the medium or biological tissue (reaction **4**), and reaction of the cyclized form with uncyclized tertiary mustard (3°) to give a number of dimers or polymers (reaction **5**). Reactions **3** and **4** are the most common, with reaction **4** having the greater biological significance (Golumbic, 1946; Fruton, 1946a,b).

In a previous section, we showed that an additional methyl group on quaternary piperidine derivatives causes significant lowering in muscarinic activity. Instead of those two methyl groups on quaternary piperidine, our proposed spiropiperidine derivatives possess two methylene groups which form the highly strained three membered aziridinium ring with the bridged nitrogen atom. Therefore, it would be possible that the high reactivity of the

aziridinium ring can compensate somewhat for any lowered muscarinic activity resulting from the steric hindrance due to the additional methylene group on the nitrogen.

C. Preliminary Target Molecules

The studies described above encouraged us to set our target molecules as shown in Fig. 10. The additional methyl group on aziridinium ring also was suggested to study the stereochemical environment of an anionic locus at the receptor site. Although, it was expected that maintaining the stereochemistry at the chiral center on the diastereoisomeric aziridinium compounds (**33** - **36**) would be difficult, it was a very interesting challenge to install the various stereocenters using different methodologies.

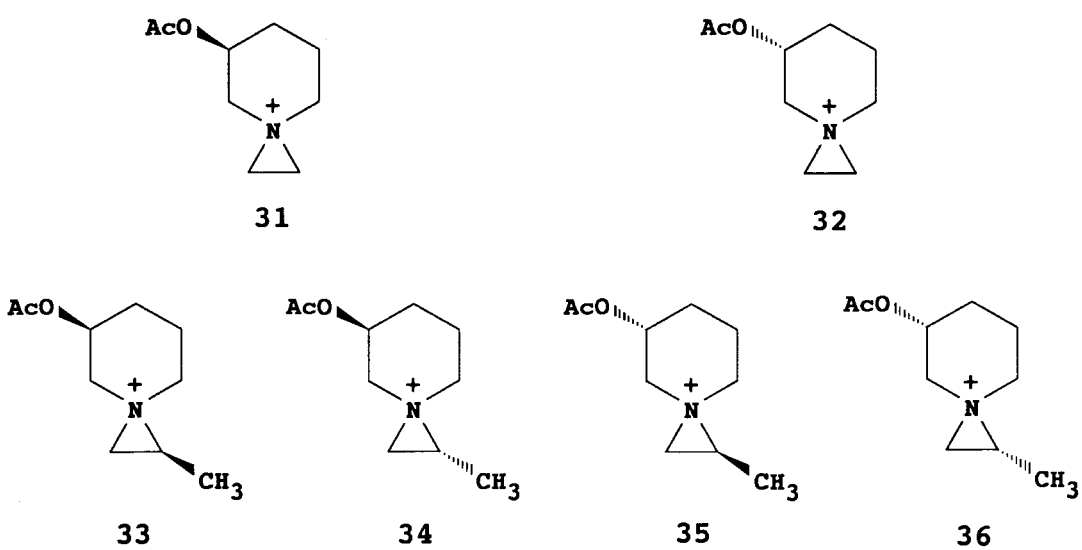


Figure 10. Preliminary target molecules

CHAPTER IV

RESULTS AND DISCUSSION

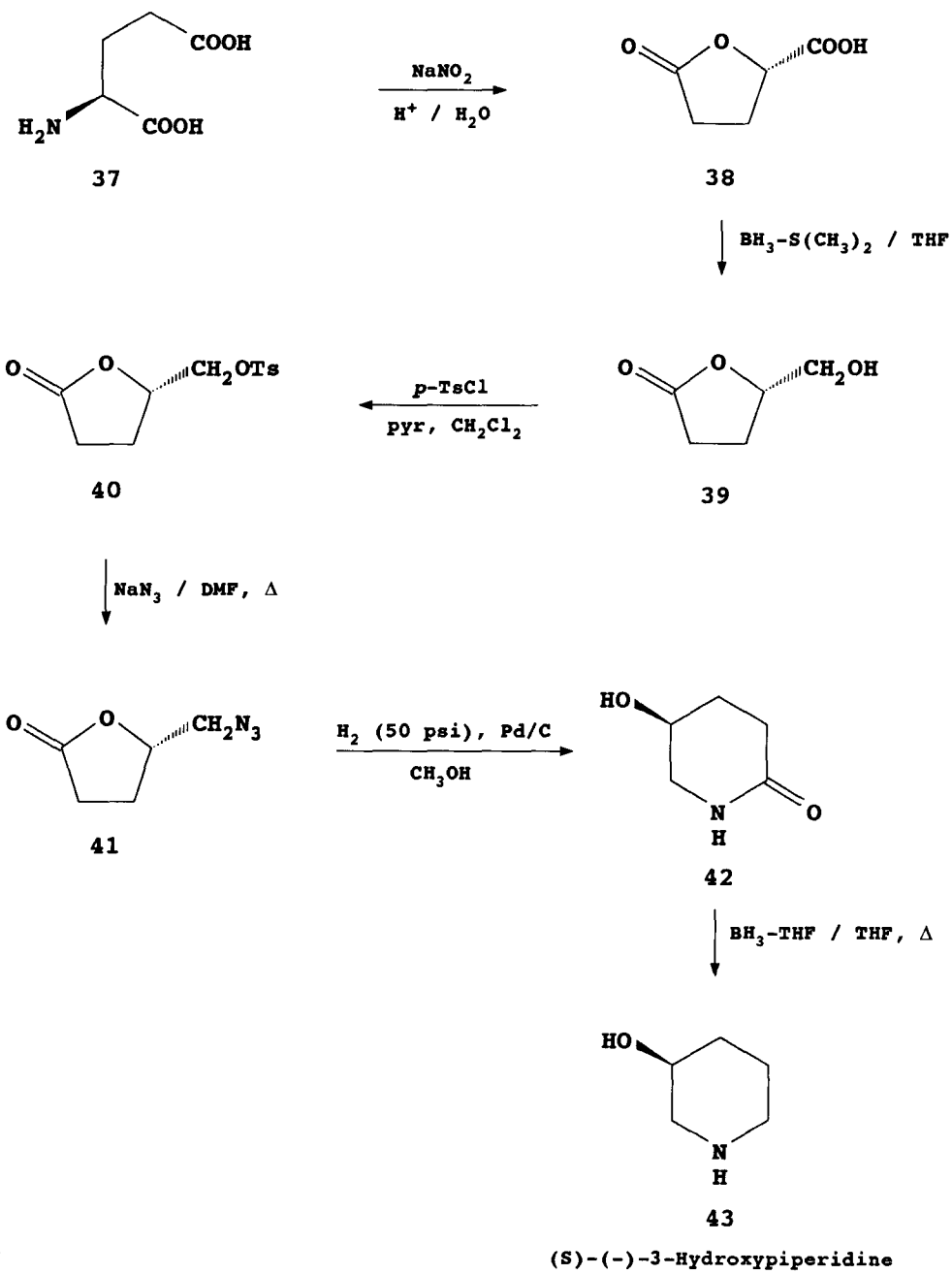
Summary: Synthesis of the precursors to target molecules (**31-36**) was explored by two different routes. First, we studied a synthetic pathway by way of the chiral 3-hydroxypiperidines, **43** and **70**, which were synthesized from the optically pure enantiomers (S)-glutamic acid **37** and (R)-glutamic acid **64** (Aldrich), respectively. We also used fractional recrystallization as a pivotal step toward the preparation of **31 - 36**, using a chiral resolving agent to first separate the enantiomers of the 3-hydroxypiperidine from a commercially available racemic mixture (Fluka). This fractional recrystallization pathway was performed to assure that the enantioselectively synthesized chiral (S)- and (R)-3-hydroxypiperidines were enantioenriched without undergoing significant racemization during synthesis by comparing the physical properties of those piperidinols produced from each pathway. In section A, we will show how we approached to our target molecules by enantioselective synthetic strategy. In section B, we will describe the parallel route in which we used a fractional recrystallization method (Sievertsson *et al.*, 1972; Lambrecht, 1976a,b).

A. Enantioselective Synthesis

1. *Preparation of (S)-(-)-3-Hydroxypiperidine*

Although several research groups have utilized fractional recrystallization of a racemic mixture of 3-hydroxypiperidine to access their chiral piperidine derivative compounds, few literature syntheses of (S)-(-)-3-hydroxypiperidine **43** have been reported (Deane and Inch, 1969; Olsen *et al.*, 1985). In connection with our studies toward the synthesis of the possible chiral neurotoxins (**31-36**), which contain the 3-acetoxypiperidine unit with *S* or *R* configurations, we were interested in designing the synthetic pathway by way of constructing the key intermediate **43**. We have succeeded in preparing (S)-(-)-(**43**) in six steps (Scheme II) in approximately 31 % overall yield from the commercially available, natural amino acid, (S)-(+)-glutamic acid **37**. The individual steps of this synthesis are discussed next.

Scheme II. Preparation of (S)-(-)-3-Hydroxypiperidine



In a typical diazotization procedure, (S)-(+)-glutamic acid **37** was treated with 2 M HCl aq. solution and sodium nitrite resulting in the crude lactone carboxylic acid (S)-(+)-(**38**) with retention of configuration. The procedure first results in the formation of a diazoyl group at C_2, which lactonizes rapidly. The purification of (S)-(+)-(**38**) by single solvent (chloroform) crystallization afforded colorless, needle shaped crystals. However, the high yield (72 %) obtained by Doolittle's group (1984) was not obtained, and usually 40-60 % was isolated. In the familiar reaction of primary amines with nitrites and acid, the species that is acting as the effective nitrosating agent has been shown to depend on the conditions although it is apparently never HNO_2 itself. At low acidity N_2O_3 (ONO-NO) is thought to be the predominant nitrosating reagent according the following equilibrium equation (Eq. 2):

$$2HNO_2 \rightleftharpoons ONO\text{-}NO + H_2O \qquad (2)$$

As the acidity increases, the protonated nitrous acid, H_2O^+-NO and finally the nitrosonium ion ^+NO are believed to be reasonable nitrosating agents along with nitrosyl chloride, NOCl when HCl is used as acid. The general mechanism of formation of diazonium salt can be described as shown in Fig. 11.

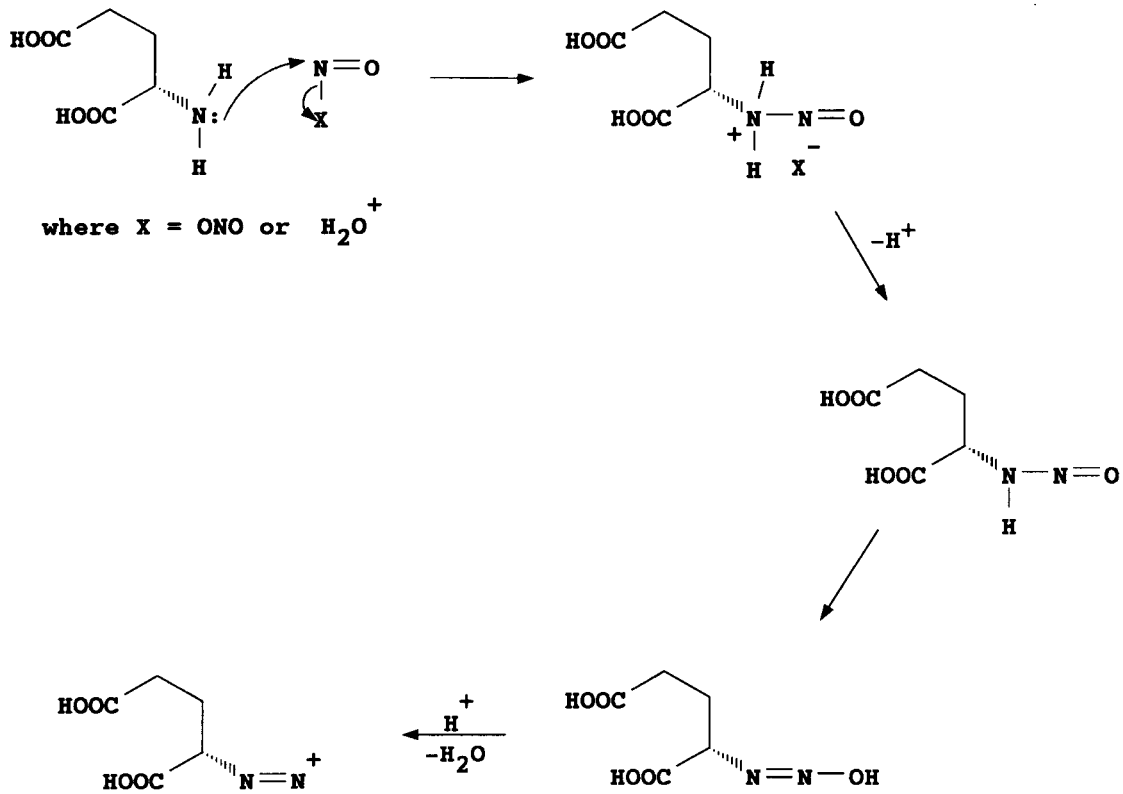


Figure 11. Formation of diazonium salt

Formation of the γ-butyrolactone carboxylic acid (S)-(+)-(**38**) with retention of configuration can be interpreted by two possible mechanisms; 1) intramolecular participation by α-carboxylic group allows formation of an α-lactone intermediate, which is subsequently ring opened by the nucleophilic γ-carboxylic moiety (Fig. 12), or 2) since the reaction was conducted in aqueous solution, water can first substitute the nitrogen leaving group and this

hydronium intermediate is then displaced by the γ-carboxylic hydroxyl group (Fig. 13).

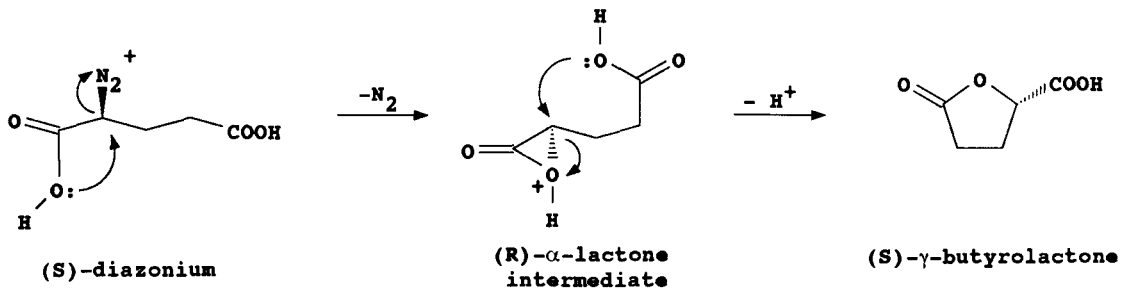


Figure 12. Formation of γ-butyrolactone via α-lactone intermediate

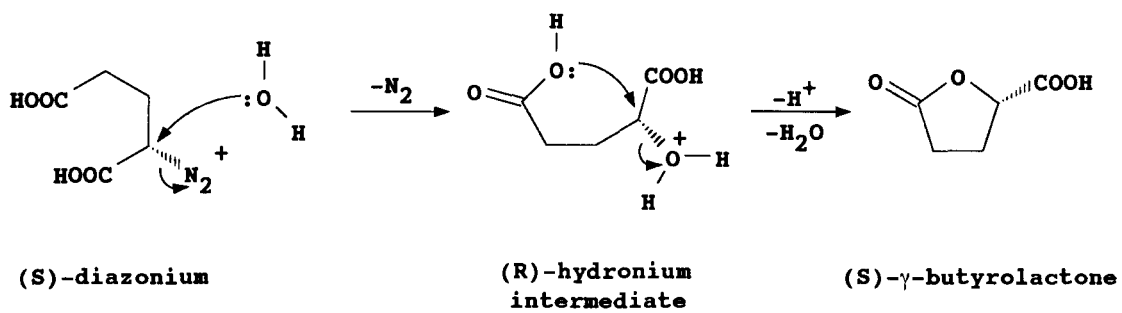
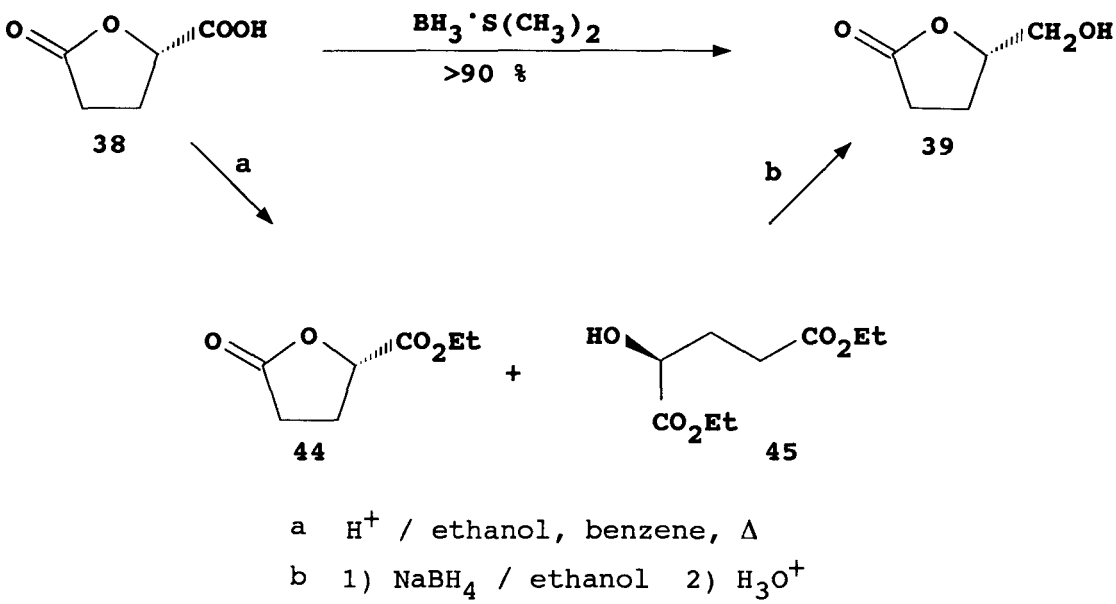


Figure 13. Formation of γ-butyrolactone via hydronium intermediate

Both mechanisms involve consecutive inversions of configuration resulting in retention of the original configuration. The lower yield possibly was caused by the interference of the acidic reaction conditions in which the relatively strong base, the primary amine group of glutamic acid, will be protonated leading to

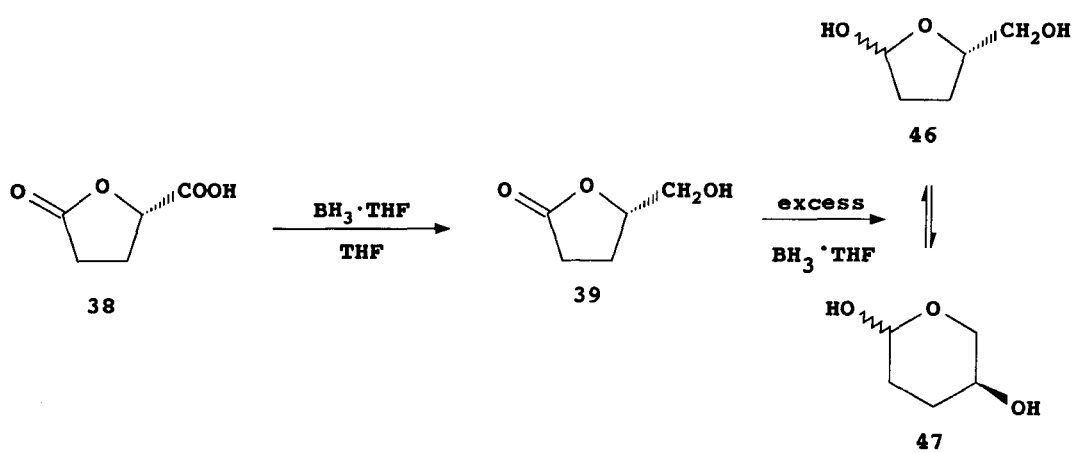
reduced nucleophilicity. Taniguchi *et al.* (1974) converted this lactone carboxylic acid **38** to the corresponding lactone ethyl ester **44**, and reported that the purification of this ester by vacuum distillation caused partial racemization at the high boiling point (Scheme III).

Scheme III. Synthesis of (S)-(+)-γ-Hydroxymethyl-γ-butyrolactone



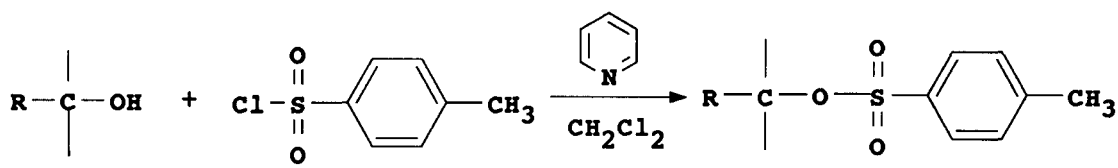
Surprisingly, they could reduce the lactone ethyl ester (S)-(+)-(**44**) to the primary carbinol (S)-(+)-(**39**) with $NaBH_4$. $NaBH_4$ is most known as a mild reducing reagent that is reluctant to reduce the ester group. Later, Ravid *et al.* (1978) studied this step more closely and found that the over-esterified side product (S)-(-)-(**45**) was produced along with the desired product **44**, but they did not mention the ratio of these two products. Therefore, it is believed that the racemization mentioned by Taniguchi's group possibly resulted from

contamination by this over-esterified impurity **45** (see the sign of the rotation). Without further separation, both **44** and **45** were reduced together with $NaBH_4$ to yield (S)-(+)-(**39**). On the other hand, they found a more convenient direct reduction of the carboxylic acid **38** using borane-methyl sulfide to give high yields of **39** following distillation. The latter reagent was selected for our use, and (S)-(+)-γ-hydroxymethyl-γ-butyrolactone **39** was prepared in 90-92% yield following purification by flash column chromatography using methanol/chloroform (4:96) as the eluting system. ^{1}H and ^{13}C NMR spectra of **39** were identical to the previous reports and the rotation was satisfactory. We also tried reduction of the lactone carboxylic acid with 1 M borane-tetrahydrofuran complex in THF (Yoon *et al.*, 1973). This stronger reducing agent, however, resulted in overreduction to afford an equilibrium of two lactols **46** and **47** which could be observed by TLC (stained with *o*-anisaldehyde with heating).



Consequently, lower yields of **39** were acquired using BH_3•THF complex in THF. The lactone alcohol **39** gave a R_f = 0.17 while lactol **46** (2-hydroxy-5-(S)-hydroxymethyl tetrahydrofuran) showed a R_f = 0.09, and the δ-lactol **47** showed a R_f = 0.20. Clearly, the chromatographic complexity further reduces interest in the use of this reducing agent.

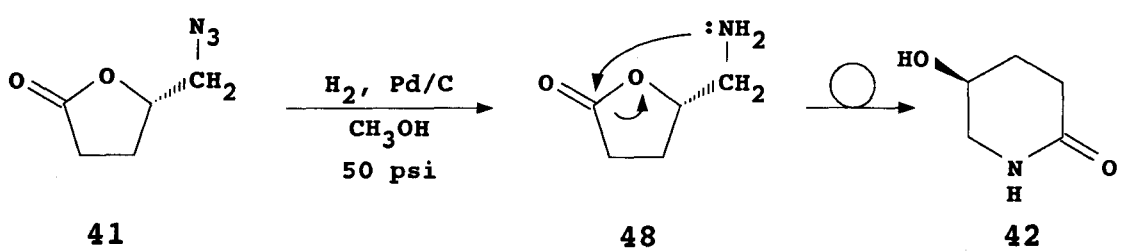
The tosylation (sulfonylation) of alcohols is a common transformation that is often used to facilitate subsequent nucleophilic substitution reactions.



Mori (1975) tosylated the lactone alcohol **39** with *p*-toluenesulfonyl chloride in pyridine (48 %), which was a general condition for tosylation of alcohols at that time. Ho and Davies (1983) modified and succeeded in obtaining the (S)-(+)-tosylate **40** in higher yield (96 %) by using methylene chloride as solvent. Kabalka's group (1986) reported that the highest yields of pure tosylates based on starting alcohol (mostly primary alcohols) are obtained using a 1 : 1.5 : 2 ratio of alcohol/tosyl chloride/pyridine in chloroform. They showed that pyridinium salts were generated in routine tosylation reactions in which pyridine is used as a base resulting in a concomitant loss of the desired tosylate product during workup. Olsen *et al.* (1985) modified the tosylation of

the lactone alcohol **39** by using a catalytic amount of N,N-dimethylamino-pyridine (94 % yield). Using Ho and Davies' method, we obtained tosylate **40** (Scheme II) in comparable yield (93 %). The absence of hydroxyl group was recorded on IR and the two methylene protons are shown as an AB_q pattern (4.13 ppm; ddd, J = 4.18, 8.69, and 11.0 Hz).

Displacement of the sulfonate group with sodium azide in N,N-dimethyl-formamide gave 5-azido-4-pentanolide **41** in 93 % yield. The N_3 stretching was recorded on IR (2120 cm^{-1}) and this azide compound **41** shows very weak UV activity on TLC analysis. The transformation of **41** into (S)-(-)-3-hydroxypiperidine **43** in two steps had been reported by Deane and Inch (1969). This report, however, contained no experimental details or yields. Olsen *et al.* (1985) described these steps in a somewhat advanced experimental section. The catalytic reduction of the lactone azide **41** generates the intermediate primary amine **48** (Fig. 14), which expands the size of the ring via intramolecular nucleophilic attack at the lactone carbonyl to form the powdery crystals of (S)-(-)-5-hydroxy-2-piperidinone **42** in 96 % yield (mp 120-121 °C; lit. 121-122 °C).



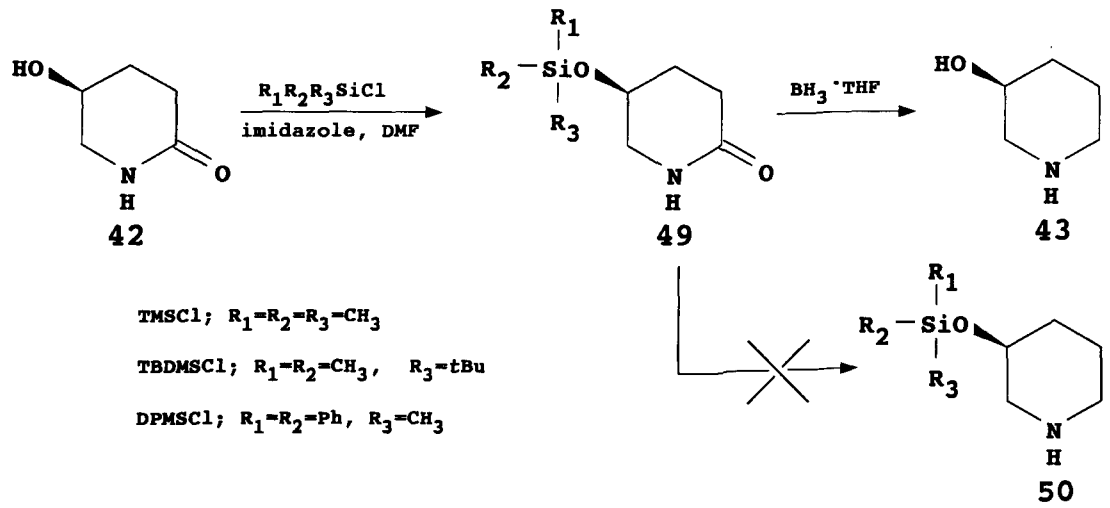
41 **48** **42**

Figure 14. Ring expansion via primary amine

The new chiral center shows the same absolute configuration, but the sign of rotation of this lactam **42** changed from (+) to (-).

Reduction of lactam carbonyl moieties had been reported by Corey's group (1975) using $LiAlH_4$ (again, no experimental details or yields) and Olsen's group (1985) who utilized borane tetrahydrofuran complex. We tried to reproduce both methods but could not obtain a satisfactory yield of the piperidinol **43** (< 60 %). It is believed that the high solubility of the product 3-hydroxypiperidine (**43**) in water (1 g / 10 mL) possibly caused low recovery during aqueous workup. The extremely highly polar amino alcohol **43** was purified by flash column chromatography using methanol/chloroform (3:7). Due to the use of high ratios of methanol as eluting solvent, some contamination of the product with silica gel resulted which caused broadening of ^1H NMR peaks. Filtration through a Celite pad enhanced the purity of the compound, but significant loss of yield resulted. Only rotation value and melting point were reported in the literatures; however, the ^1H NMR shows a spectrum consistent with the structure.

Until racemic 3-hydroxypiperidine became commercially available, we had struggled to identify this highly polar compound **43** by TLC. To circumvent this problem, we imposed a high molecular weight protecting group on the hydroxyl group of the lactam **42** with or without a chromophore to aid visual detection on TLC and/or reduce the polarity of the desired product **43**. Silane reagents such as *tert*-butyldimethylsilyl chloride (TBDMSCl),



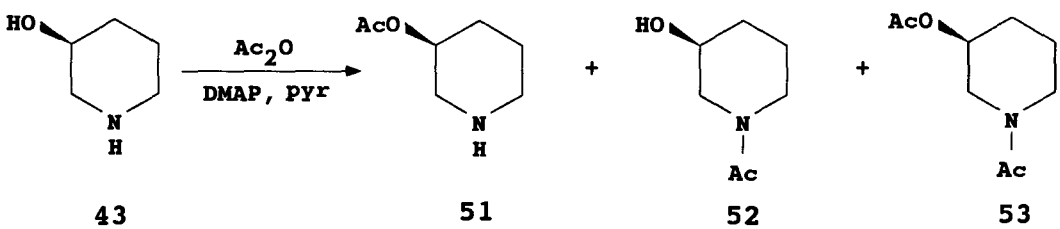
diphenylmethylsilyl chloride (DPMSCl), and trimethylsilyl chloride (TMSCl) were used to protect the hydroxyl group on the lactam **42**.

The methyl groups attached to the silane appeared at 0.1 ppm and, except the TMSCl protection, strong UV activities induced by the phenyl groups were detected on TLC analysis. Reduction of the cyclic amide group was next attempted with BH_3•THF reagent. However, it was found that the borane also removed the protecting group as well as reducing the amide function, which led back to **43**. Thus, a significant portion of our investigation became dedicated to finding a protecting group strategy that would nearly furnish the target precusors.

2. *Synthesis of (S)-(-)-3-Acetoxypiperidine - A Divergent Intermediate Molecule*

After the key intermediate **43** was synthesized, studies were continued to obtain the divergent molecule (S)-(-)-3-acetoxypiperidine **51**. With **51** in hand, an array of aziridinium precursor functional group could be installed. First, we simply tried the direct approach; acetylate the hydroxyl function with acetic anhydride and pyridine in the presence of catalytic amount of N,N-dimethylaminopyridine (Scheme IV).

Scheme IV. Acetylation of (S)-(-)-3-Hydroxypiperidine

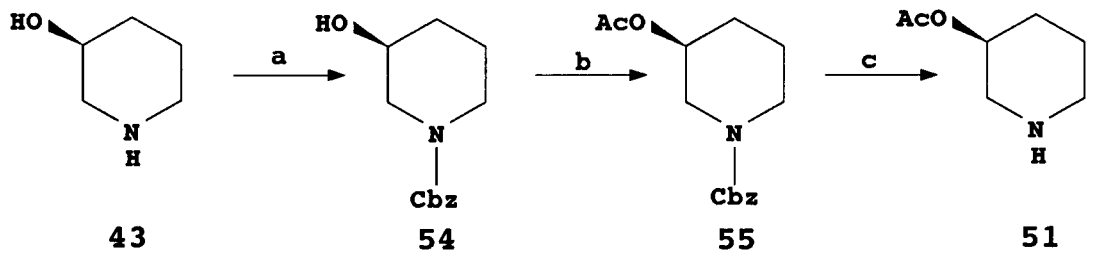


43 **51** **52** **53**

However, although the desired product, O-acetylated **51**, was obtained, the expected competition between the hydroxyl group and the cyclic secondary amine resulted to give N-acetylated **52**, and diacetylated **53** side products which were recorded on ^{1}H NMR by showing two different chiral protons at C_3. The chiral proton of **52** is shown at the similar region to the proton of 3-hydroxypiperidine **43** (3.6-3.7 ppm) while the chiral protons of **51** and **53** are shown at further downfield (4.9-5.0 ppm). In an attempt to reduce reaction at

the secondary amine, the acetylation was conducted under acidic conditions with the expectation that protonation at the amine would first occur thereby reducing its reactivity. Despite all attempts with acid conditions, mixtures of acetylated products were obtained. To circumvent this problem with the amine moiety, we protected the amine function using carbobenzyloxy chloride (Cbz-Cl) (Scheme V).

Scheme V. Synthesis of (S)-3-Acetoxypiperidine via Protecting Amine Moiety



43 54 55 51

a; Cbz-Cl / $NaHCO_3$, toluene b; Ac_2O / DMAP, pyridine c; H_2(35 psi), Pd/C, CH_3OH

The protecting studies were performed first with commercially available racemic 3-hydroxypiperidine (Fluka), and gave an outstanding yield (90-95 %). Similar results were achieved with the chiral (S)-(-)-hydroxypiperidine **43** to yield **54** (~90 %). An ^1H NMR study of this amine-protected intermediate **54** showed a very poorly resolved spectrum, and it is believed that this poor resolution resulted from equilibration of the highly flexible protecting group. However, removal of the Cbz group yielded (S)-(-)-(**43**) which indicates the

protecting process was successful. Taking advantage of the successful result from the protecting study, in order to prevent significant loss of **43** during the purification process, we conducted the following two steps, namely, the reduction of lactam and the amine protection, *in situ* without further purification of **43**. The overall yield of **54** from the lactam **42** was 76%, which implies that, considering the yield of protecting step (90 %), we recovered more than 80 % of **43** without significant loss (after purification, the yield of **43** was < 60 %, and this chiral piperidinol was contaminated by silica gel). Conversion of the hydroxyl group (Scheme V, step b) to acetoxy compound **55** was now achieved successfully (85 %) using acetic anhydride, pyridine and a catalytic amount of DMAP under anhydrous conditions. Again, resolution of the ^{1}H NMR was extremely poor due to the flexibility of the protecting group; however, a successful elemental analysis result indicates that acetylation proceeded.

The removal of Cbz group (Scheme V, step c) was conducted under balloon pressure. Unfortunately, it was found that the free base **51** was somewhat unstable forming either the N-oxide, and/or underwent transacetylation (Fig. 15) resulting in N-acetyl-3-hydroxypiperidine. This latter side reaction predominates after long hydrogenolysis reaction times (usually, stirring overnight under balloon pressure). Therefore, in order to shorten the period of reaction time, we performed this step on a Parr Hydrogenator, using 10 % palladium on active carbon as a catalyst, under 35

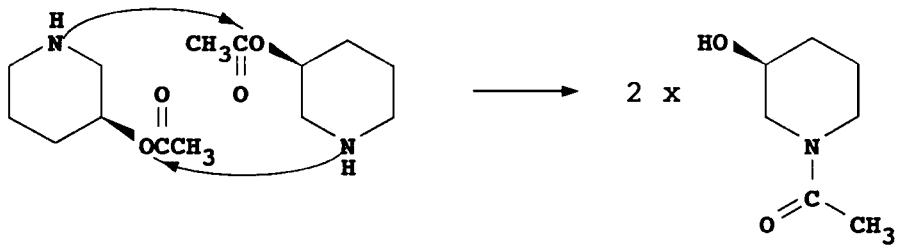
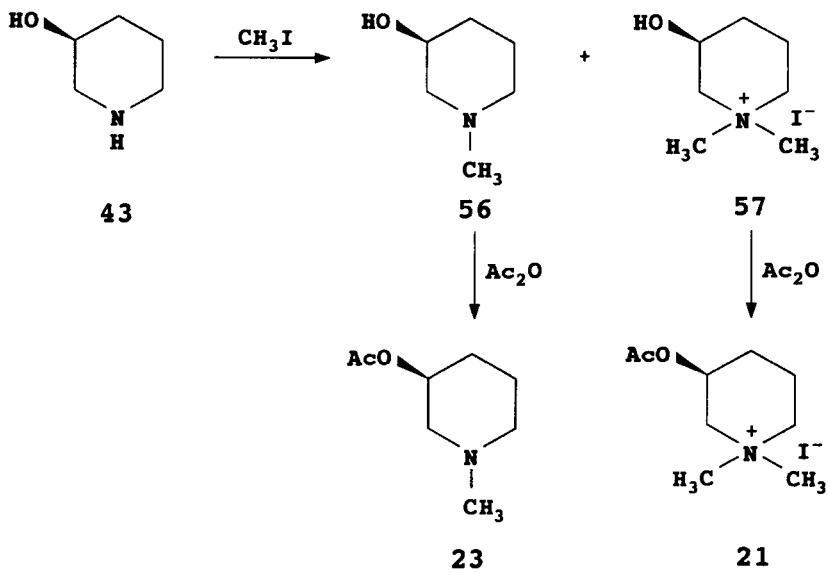


Figure 15. Transacetylation between two (S)-(-)-3-acetoxypiperidines

psi (slightly higher than 1 atm) of hydrogen with which the reaction completed within three hours (monitored by TLC). Purification of **51** was achieved by flash column chromatography using chloroform/methanol (9:1) as eluting system to yield a pale yellow liquid **51**. The free base, secondary amine proton appears at 7.96 ppm as a singlet, and removal of the Cbz group shows a better resolved spectrum.

In Chapter III, it was described that Lambrecht and Muschler synthesized and investigated the muscarinic activities of N-methyl-3-acetoxy-piperidine **23** and N,N-dimethyl-3-acetoxypiperidinium iodide **21**. In their synthetic method, alkylation of the amine was achieved first by reacting the chiral 3-hydroxypiperidine with methyl iodide, and later, esterification was managed on the hydroxyl group as shown in Scheme VI.

Scheme VI. Synthesis of (S)-N-Methyl-3-acetoxypiperidine and (S)-N,N-Dimethyl-3-acetoxypiperidinium Iodide



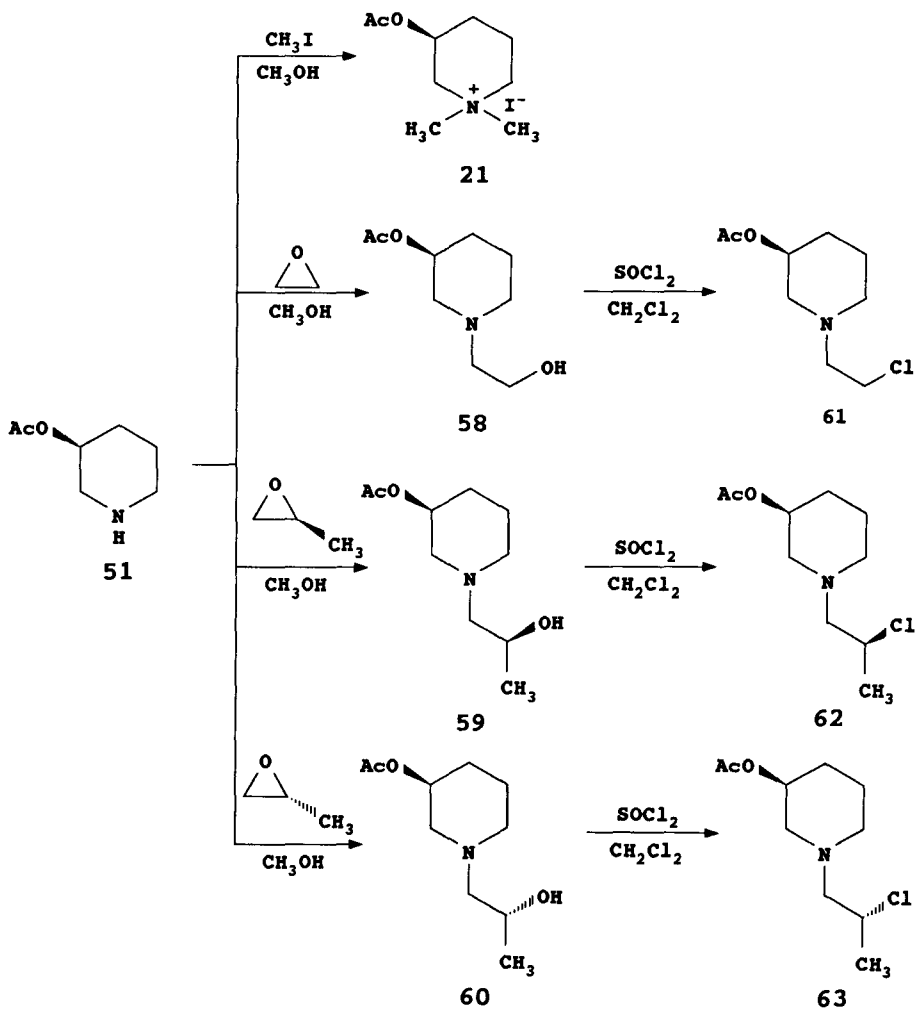
43 56 57 23 21

During construction of the β-hydroxyalkyl-piperidine derivatives, we recognized that conducting the alkylation before esterification would cause difficulties in controlling selective esterification on the hydroxyl group of the piperidine ring while the other hydroxyl group on the N-alkyl chain must remain unreacted in preparation for the next step (chlorination). Hanin's group managed a very similar step by stopping the acetylation at the point the diacetylated product began to form (Chapter III, Scheme I). However, low yields were reported. Therefore, in conclusion, it was a very advantageous idea that we prepared the divergent intermediate **51** via protecting the amine group with Cbz-Cl, since selective functionalization of one hydroxyl group at a time was managed.

3. *Alkylation of (S)-(-)-3-Acetoxypiperidine and Synthesis of β-Chloroalkyl-3-acetoxypiperidines*

Several alkylating reagents were planned to react with the divergent intermediate **51** to eventually obtain the N-(β-chloroalky)piperidines, the precursors of our aziridinium target molecules (Scheme VII).

Scheme VII. Synthesis of N-(β-Chloroalkyl)-(3S)-acetoxypiperidines



Methylation of (S)-(-)-(**51**) with methyl iodide was sucessfully achieved to yield (S)-(-)-N,N-dimethyl-3-acetoxypiperidinium iodide **21** (65 %). An excess amount of methyl iodide was used to ensure that the reaction was completed without leaving any monoalkylated product **23**. An ^{1}H NMR study indicated that the two methyl groups on the nitrogen are not located in the same chemical environment (Fig. 16) showing two methyl peaks with a 0.05 ppm difference at chemical shifts 3.50 and 3.55 ppm. However, the chemical shift difference is not very large, possibly indicating that the 1,3-diaxial interaction between N-methyl and acetoxy groups is not significant or ring flip leads to a time averaged equilibrium. This reduced interaction results from the fact that the acetoxy moiety may assume a conformation pointed away from the N-methyl group.

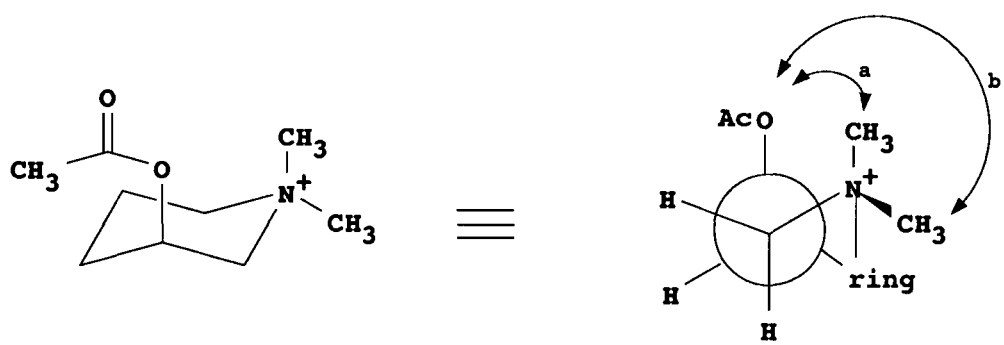
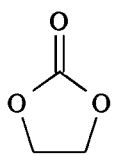
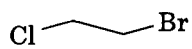
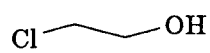
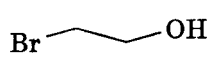


Figure 16. Two different environments for methyl groups of the N,N-dimethyl-3-acetoxypiperidine **21**. The -CH_3(a) shows interaction with the axial acetoxy group appeared at slightly further down field (3.55 ppm), while the -CH_3(b) is away from the acetoxy group resulted in upper field (3.50 ppm) in ^{1}H NMR spectrum.

Synthesis of the (S)-(-)-N-(2-hydroxyethyl)-3-acetoxypiperidine **58** was approached by a variety of methods as shown in Table XI. First, reaction with

Table XI. Reagents and yields used for synthesis of (S)-(-)-N-(2-hydroxyethyl)-3-acetoxypiperidine **58**.

Reagent	Reaction condition	Yield
 ethylene carbonate	CH_3OH	no reaction
	K_2CO_3/CH_3OH	~ 10 %
 1-bromo-2-chloroethane	NaI / CH_3CN, 40 °C	23 % [a]
	NaI / Et_2O, CH_2Cl_2 (2:1)	15.5 % (77.5 %) [a, b]
 2-chloroethyl alcohol	DMF	< 10 %
 2-bromoethyl alcohol	NaI / THF	11.4 %
	TEA, CH_2Cl_2	40.6 %
	K_2CO_3/ H_2O	18 %
	NaOH / H_2O	38 %

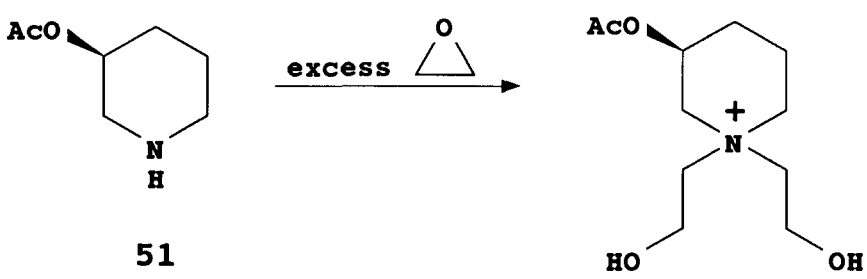
a : yielded N-(2-chloroethyl)-3-acetoxypiperidine
b : 77.5 % yield was calculated based on the amount of consumed starting material, about 80 % of unreacted starting material was recovered.

2-haloethyl alcohols were studied for this alkylation step. However, the yield of reaction of **51** with 2-bromo- or 2-chloroethyl alcohol in DMF, H_2O, or THF was not satisfactory (~20 %). Unfortunately, reaction of **51** with ethylene carbonate in anhydrous methanol did not show evidence of alkylation; however, some reaction proceeded in the presence of potassium carbonate.

Testing the reactivity of this cyclic secondary amine **51** with epoxides was initiated by using a racemic mixture of propylene oxide. Even though separation of the diastereoisomers seemed to be impossible (overlapping spots of the two diastereoisomers on TLC), the yield of this reaction in H_2O or methanol showed remarkable enhancement. Therefore, before the commercially available ethylene oxide (Fluka) was purchased, we tried to first generate the ethylene oxide *in situ* by stirring 1 : 1 equivalent amount of 2-bromoethyl alcohol and sodium hydroxide in water at 0 °C and manage the alkylation with **51** in aqueous media. Problems arose in isolation due to increased solubility of the primary alcohol **58** in the aqueous reaction mixture. After removal of most of the water under reduced pressure, the concentrated residue was chilled at - 78 °C. Using a vacuum pump, the frozen, last trace of water could be removed taking advantage of low heat of sublimation of ice (freeze drying) under a extremely low pressure, to give a yellow colored liquid residue which was diluted with ethyl acetate followed by drying over Na_2SO_4. Purification by column chromatography afforded the pure alcohol **58** with somewhat low yield (mostly 35 - 40 %). Two sets of methylene protons of N-

alkyl moiety was shown at 2.47-2.51 and 3.53-3.56 ppm as triplets on the ^1H NMR. The low yield might have been caused by the uncertain amount of the ethylene oxide produced *in situ*, or the oxirane molecules were polymerized under the basic aqueous conditions. It also could be possible that while the last trace of water was being removed under vacuum pump pressure, the protic solvent molecule could carry the product or this alcohol product could be evaporated coincidently.

The direct use of commercially available ethylene oxide still did not afford a reasonable yield when the reaction was conducted in water as the reaction solvent system. Changing the solvent to methanol gave very consistent results with much higher yield (~ 60 %) due to the simple workup. The reaction had to be stopped before complete consumption of the starting material because the produced tertiary amine can be over-alkylated to yield *bis*-(2-hydroxyethyl)-3-acetoxypiperidinium (below).



51

51a

***bis*-(2-hydroxyethyl)-3-acetoxypiperidinium**

To avoid ovrealkylation, oxirane gas was effused into the stirring solution of (**51**) in methanol at ice-bath temperature, then the ice bath was removed. Generally, the formation of the overalkylated product was detected when the reaction was stirred for more than 1.5 h after effusion of ethylene oxide.

Regioselective alkylation of **51** with chiral (S)-(-)- or (R)-(+)-propylene oxide (Fluka) afforded similar results to yield the diastereoisomers, (S,S)-(+)-N-(2-hydroxypropyl)-3-acetoxypiperidine **59** and (R,S)-(-)-N-(2-hydroxypropyl)-3-acetoxypiperidine, respectively **60**, when the reactions was stirred at 5 °C overnight (Fig. 17). The newly generated chiral protons of the side chain appeared at 3.72-3.81 ppm region as a multiplet. As a doublet peak, the -CH_3 group of (S,S)-isomer shows a somewhat smaller coupling constant (6.2 Hz) than that of (R,S)-isomer (8.3 Hz).

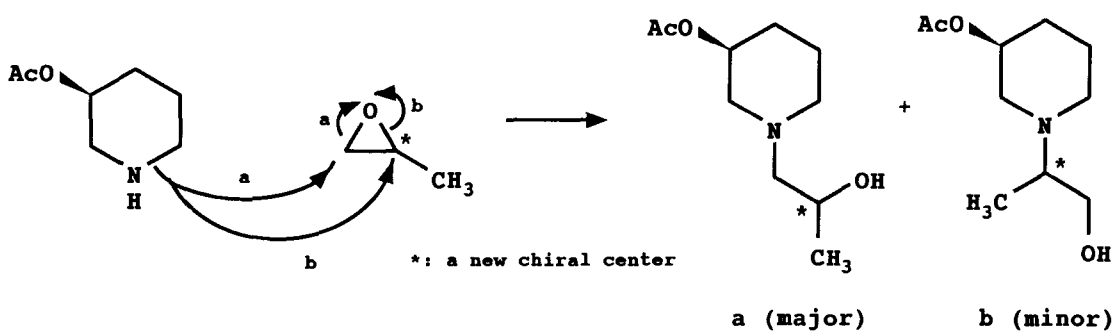


Figure 17. Regioselective nucleophilic substitution on a unsymmetric epoxide. The cyclic secondary amine will attack the propylene oxide at the carbon which has less steric hindrance (route a) preferentially with a 96 : 4 of ratio.

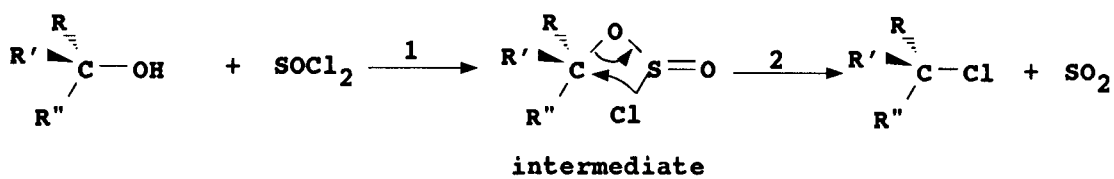
(S,S)-(+)-(**59**) and (R,S)-(-)-(**60**) are less reactive toward overalkylation

than **58**, probably, due to the increased steric hindrance of the secondary alcohol chain of the product, and the propylene oxides, so formation of the overalkylated quaternary ammonium was inconsequential. The chiral β-proton on the ethyl chain, which is located next to the hydroxyl group, was detected at 3.72 - 3.80 ppm as a multiplet peak on the ^{1}H NMR spectrum while the β-methylene protons next to the hydroxyl group of the primary alcohol **58** were shown at 3.53 - 3.56 ppm as a triplet.

Syntheses of the asymmetric mustard molecules (**61** - **63**), which are precursor molecules of the aziridinium ions, were achieved by reacting the alcohols (**58** - **60**) with freshly distilled thionyl chloride (over rinsed oil) in methylene chloride at low temperature under argon atmosphere. These β-chloroethyl (or propyl) amines (**61** - **63**) were obtained as their hydrochloride salts to prevent immediate further reaction including dimerization or, possibly, aziridinium formation. The hydrochloride salt could be converted to the free base by stirring in diethyl ether or methylene chloride with sodium bicarbonate, followed by flash column chromatography using petroleum ether and diethyl ether (1:1 or 2:1) to afford the β-chloroethyl (or propyl) amines as pale yellow liquids.

For the primary chloride compound **61**, the β-methylene protons next to the chloride appeared at 3.51 - 3.56 ppm in the ^{1}H NMR spectrum showing virtually no change in chemical shift compared to the precursor β-hydroxy molecule **58** (3.53 - 3.56 ppm). However, the α-methylene protons' chemical

shift did change from 2.47 - 2.51 ppm to 2.70 - 2.75 ppm upon conversion from alcohol to chloride. For the secondary chloride compounds **62** and **63**, the chemical shift of the chiral β-proton changed from 3.72 - 3.80 ppm to 3.95 -4.06 ppm. The doublet of the terminal methyl group of the propyl chain also showed a significant change in chemical shift from 1.08 ppm to 1.47 ppm proving that the mustard compounds were successfully synthesized. The use of thionyl chloride in the presence of a base, such as pyridine or TEA, inverts the configuration of the chiral center on the alkyl chain. As mentioned earlier, this chlorination was managed without using base control which will allow this step to proceed with retention of configuration via S_Ni mechanism. This reaction has been interpreted mechanistically as follows:



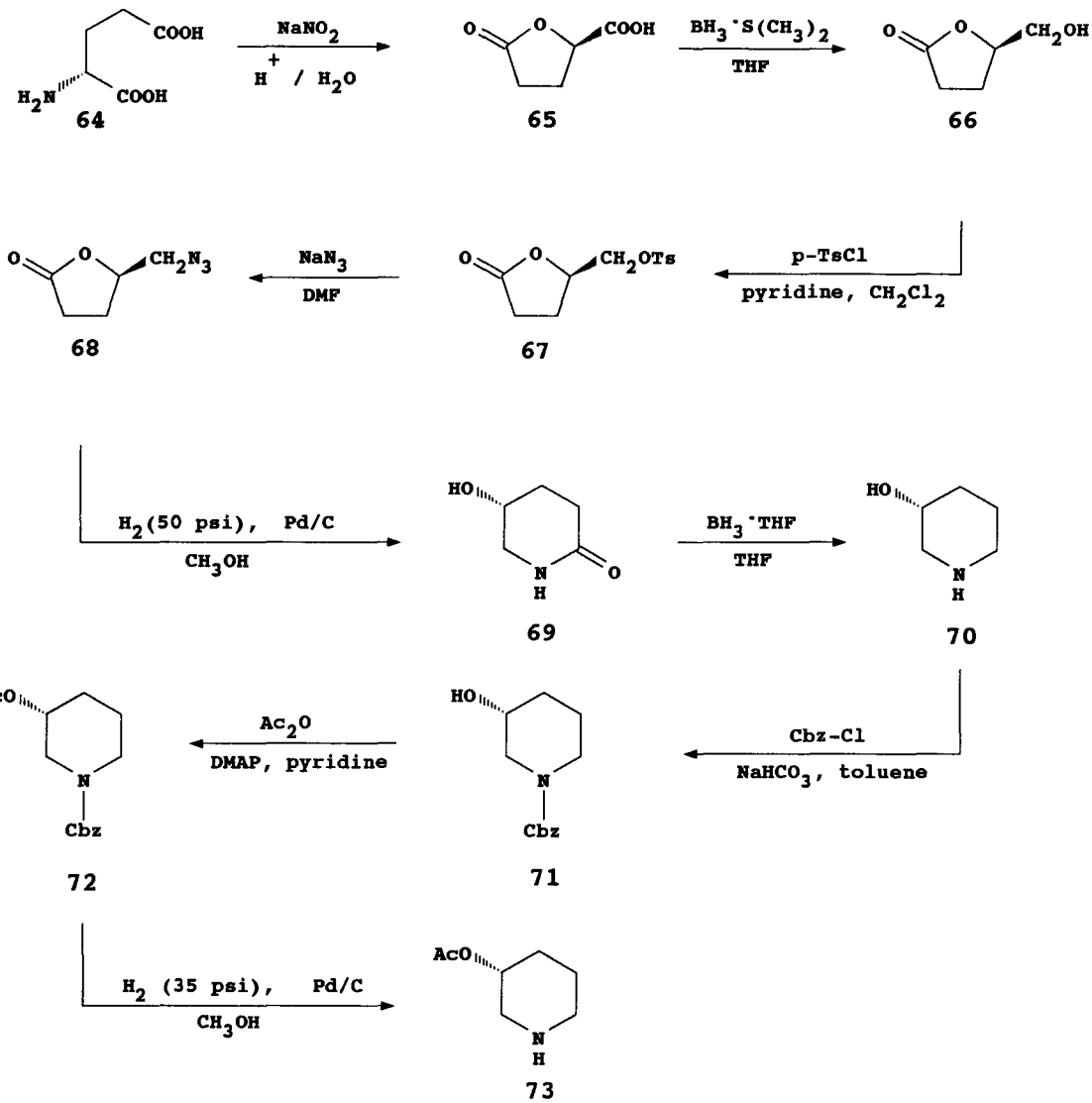
intermediate

No change in configuration can take place in stage 1 as the C-O bond is not broken. In the second stage, where this bond is broken, attack by Cl takes place from the same side of the carbon atom because of the orientation of the intermediate (Sykes, 1970).

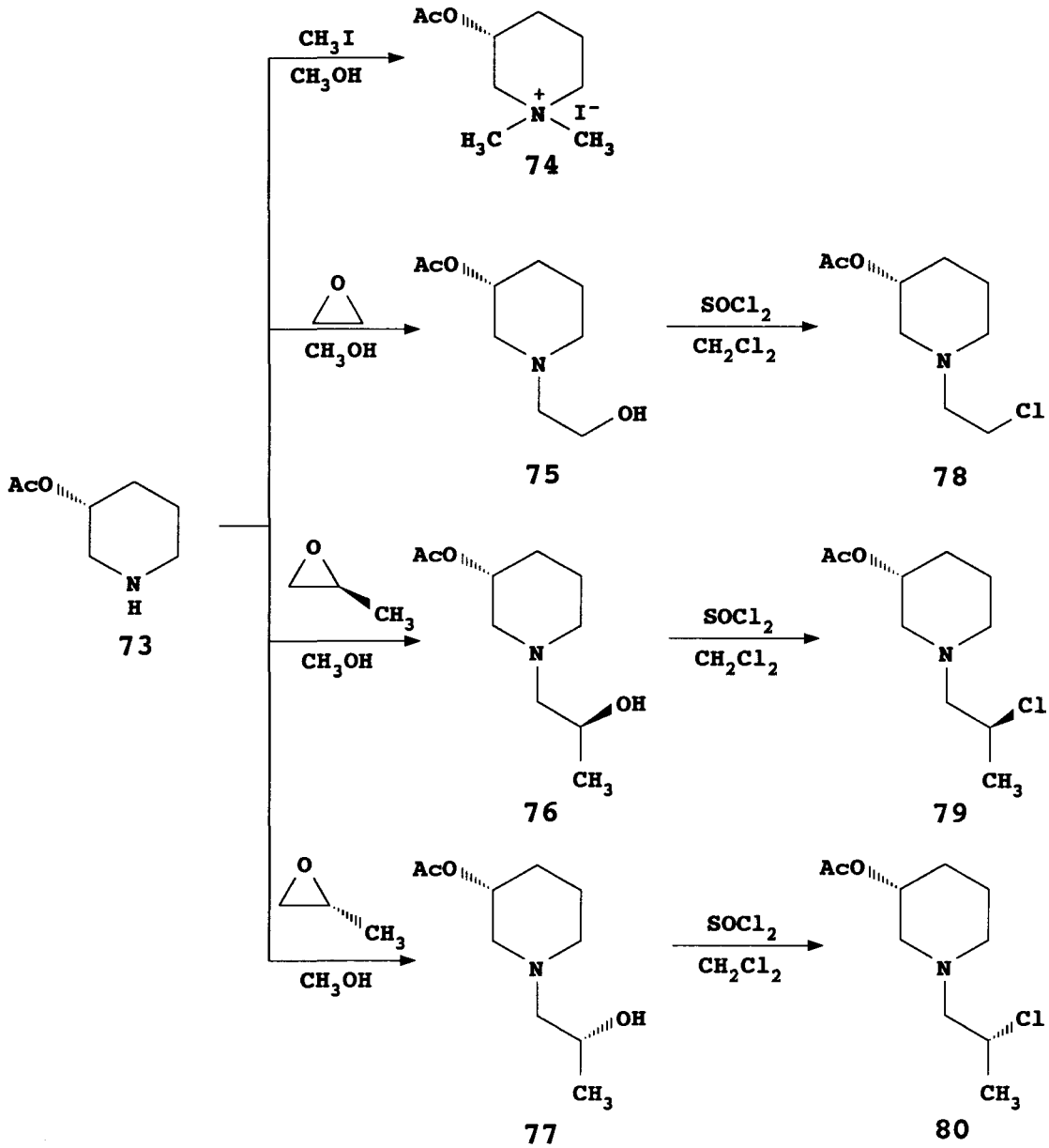
4. *Synthesis of (R)-3-Acetoxypiperidine Derivatives*

This enantiomer of (S)-3-acetoxypiperidine and its derivatives were prepared from (R)-(-)-glutamic acid **64**. The sequence is analogous to Scheme II as shown in Scheme VIII and Scheme IX.

Scheme VIII. Synthesis of (R)-(+)-3-Acetoxypiperidine



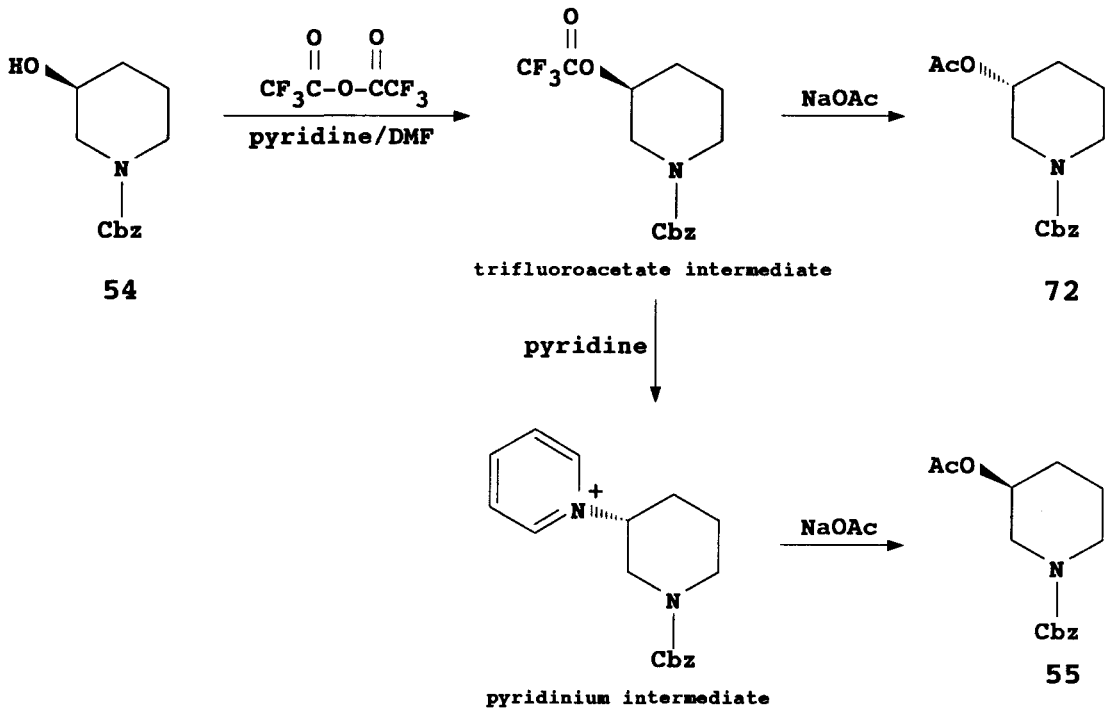
Scheme IX. Synthesis of N-(β-Chloroalkyl)-(3R)-acetoxypiperidines



As expected, unlike the natural S-(+)-glutamic acid **37**, the unnatural R-(-)-glutamic acid **64** is much more expensive starting material. Therefore, before we began the synthesis of enantiomer series of mustard compounds (**74** and **78** - **80**) from the (R)-(-)-glutamic acid **64**, we first attempted to invert the configuration of the C_3 of the (S)-(+)-N-Cbz-3-hydroxypiperidine **54**, which is obtained from the relatively inexpensive natural amino acid **37**.

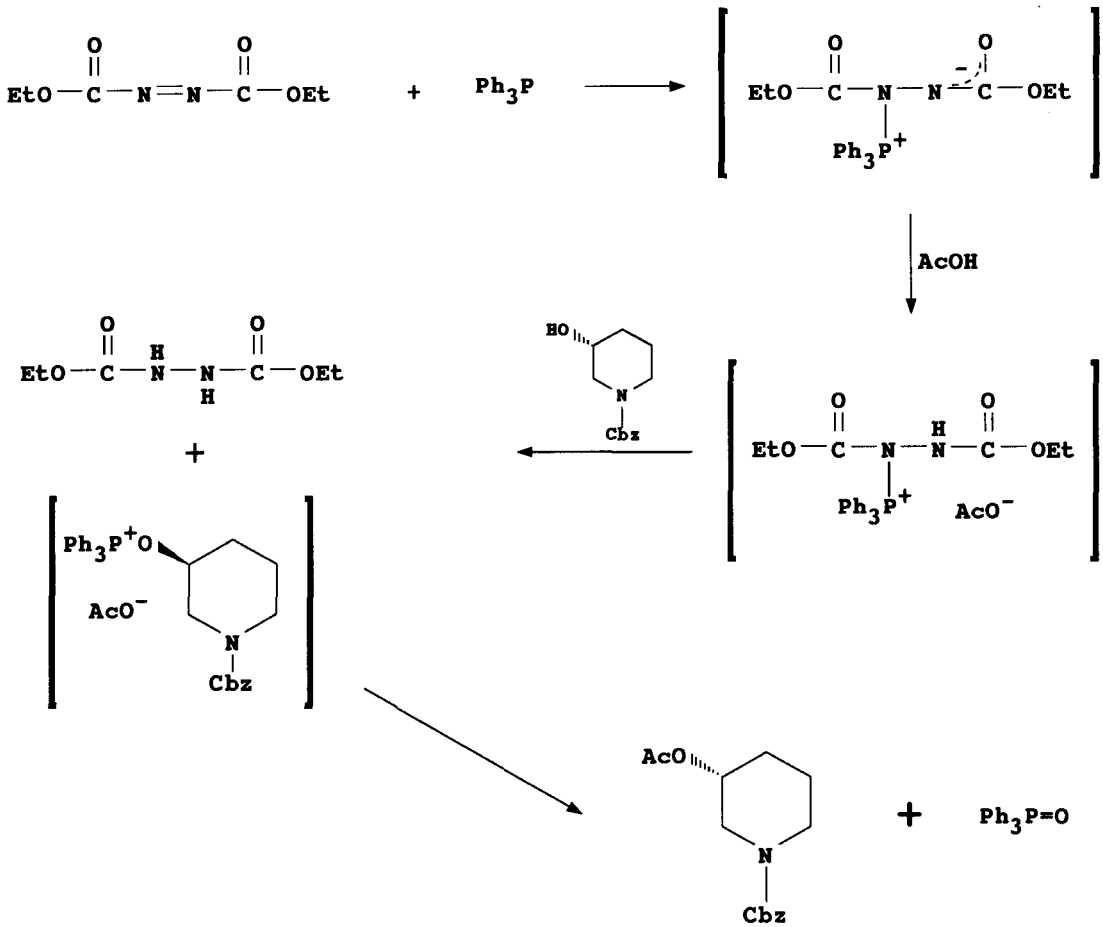
Using trifluoroacetic anhydride, transformation of the hydroxyl group of **54** into an excellent leaving group, trifluoroacetate, was attempted in the presence of pyridine as base in N,N-dimethylformamide with the hope that the trifluoracetate would be exchanged with acetate anion to obtain (R)-(+)-N-Cbz-3-acetoxypiperidine **71**, the enantiomer of **54** (Scheme X). However, as illustrated in Scheme X, pyridine first displaced the trifluoroacetate group forming a pyridinium intermediate before the acetate anion was added resulting in partial racemization of configuration. The acetate seemed not strong enough to exchange this pyridinium even at high temperature (~80 °C). More hindered base such as triethyl amine and better leaving groups (e.g., triflate, etc.) would be an ideal choice for this reaction, but further study of this system was not explored.

Scheme X. Inversion of Configuration Using Trifluoroacetic Anhydride.



The conversion of chiral alcohol into a chiral acetate with inversion of configuration using diethyl azodicarboxylate (DEAD) and triphenylphosphine was reported by Mitsunobu (1981). In Scheme XI, the mechanism of this reaction with (S)-(+)-N-Cbz-3-hydroxypiperidine **54** is illustrated.

Scheme XI. Inversion of Configuration Using Mitsunobu Conditions



An equivalent amount of glacial acetic acid and 1.27 equivalents of triphenyl phosphine and DEAD were reacted with **54** in THF, however, we could not achieve a satisfactory result (< 20%).

Having difficulties in inverting configuration at C_3 position and experiencing that this "quick way" was not easy to achieve, the author had to go back to our original strategy to obtain (R)-(+)-3-hydroxypiperidine in which the entire synthesis had to begin from the unnatural amino acid, (R)-(-)-

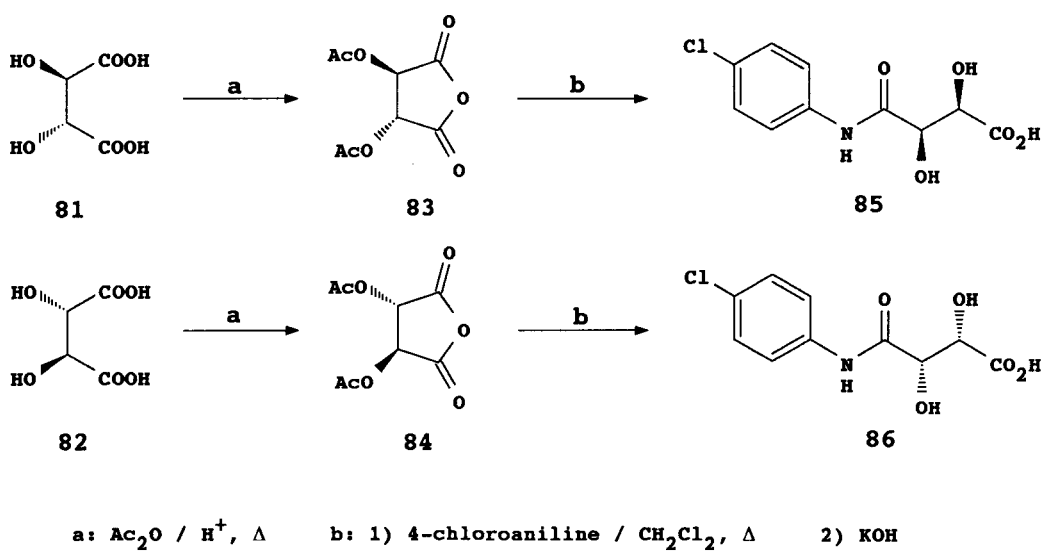
glutamic acid. However, we expected that providing enough amount of (S)-(-)- and (R)-(+)-3-hydroxypiperidines by fractional recrystallization method would compensate this time depleting synthetic strategy.

B. Fractional Recrystallization of Chiral 3-Hydroxypiperidine

1. *Preparation of 4-Chlorotartranilic Acid - A Chiral Resolving Agent*

Tartranilic acids are well known as exceptionally useful resolving agents for racemic bases. As illustrated in Scheme XII, (+)-4-chlorotartranilic acid **85** was prepared by reaction of 4-chloroaniline with (+)-diacetoxysuccinic anhydride **83** followed by basic hydrolysis of the acetyl groups first described in the procedure of Pressman *et al.* (1948). The same procedure worked fairly well with (-)-diacetoxysuccinic anhydride **84** to give (-)-4-chlorotartranilic acid **86**. The (+)- and (-)-diacetoxysuccinic anhydrides were readily prepared from the tartaric acids, (+)-(**81**) and (-)-(**82**), respectively (Rabjohn, 1963).

Scheme XII. Synthesis of Chiral 4-Chlorotartranilic Acid



(+)-Tartaric acid **81** has the *2R : 3R* absolute configuration while (-)-tartaric acid **82** shows *2S : 3S* configurations. Therefore, (+)-4-chlorotartranilic acid **85** will have *2R : 3R* configuration and (-)-isomer **86** will maintain its own *2S : 3S* configuration.

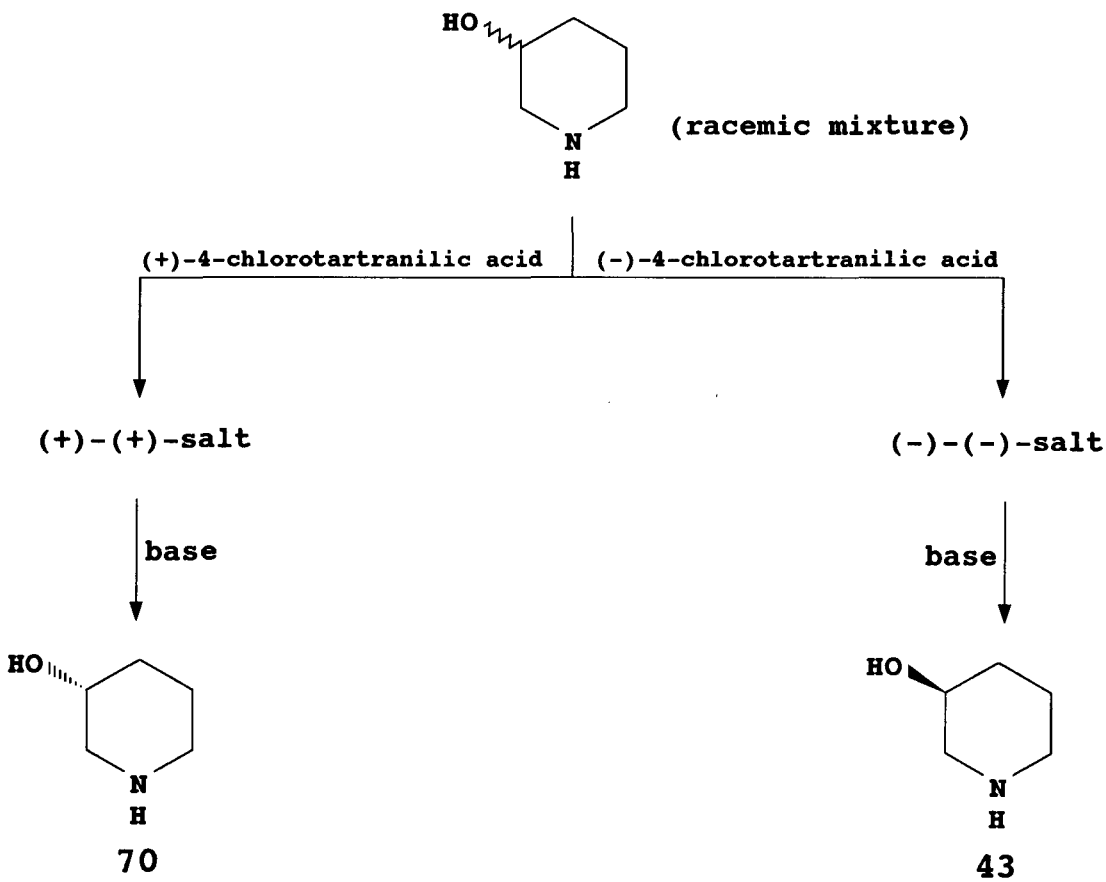
The diacetoxysuccinic anhydrides are not stable so they were prepared only as needed and converted directly to the anilide. Two acetoxy methyl groups are shown at 2.21 ppm in ^{1}H NMR spectrum while the succinic anhydride chiral protons are shown at 5.67 ppm. Both chiral resolving compounds, **85** and **86**, were recrystallized from absolute ethanol and water at 5 °C to yield colorless, needle shaped crystals (mp 192 -194 °C; lit. 193 - 195 °C).

2. *Fractional Recrystallization*

The mechanism of formation of the diastereomeric salt of a chiral 3-hydroxypiperidine with the chiral resolving agent could be interpreted by the physical/structural interactions between two chiral molecules. It is thought that, in terms of spatial arrangement, the hydroxyl groups of the chiral (+)-4-chlorotartranilic acid would interact (or repulse) with the hydroxyl group of the unfavored chiral enantiomer of 3-hydroxypiperidine ((-)-isomer). Hydrogen bonding between two molecules also could be considered as an important factor. Therefore, this physical/structural interaction results in exclusive formation of (+)-(+)-salt. With the same reason (-)-4-chlorotartranilic acid will

form a diastereomeric (-)-(-)-salt only. From a slow recrystallization at room temperature (Sievertsson, 1972), 60 - 70 % of the diastereomeric salts (needle shape) were recovered (Scheme XIII). Treatment with K_2CO_3 or $NaHCO_3$ resulted in dissociation of the acid and base to yield the chiral 3-hydroxypiperidines (pale yellowish needles, 56 - 62 %).

Scheme XIII. Fractional Recrystallization of the Racemic Mixture of 3-Hydroxypiperidine Using Chiral 4-Chlorotartranilic Acid



3. *Comparison of the Physical Properties of the 3-Hydroxypiperidines Obtained by the Enantioselective Synthesis and Fractional Recrystallization Methods*

^{1}H and ^{13}C NMR spectra of the chiral 3-hydroxypiperidines were important first determinants in the structure conformation. The other physical properties are compared as shown in Table XII.

Table XII. Physical properties of chiral 3-hydroxypiperidines from enantioselective synthesis and fractional recrystallization.

synthetic pathway	content	crystallization
56 - 61	mp (°C)	56 - 60
- 8.94 (c 4.7, CH_3OH)	$[\alpha]_D^{24}$ (°)[a]	- 8.01 (c 1.32, CH_3OH)
+ 10.45 (c, 1.0, CH_3OH)	$[\alpha]_D^{24}$ (°)[b]	+ 9.45 (c 0.65, CH_3OH)

a: (S)-(-)-3-hydroxypiperidine, b: (R)-(+)-3-hydroxypiperidine

Although the rotation values slightly deviate from each other, depending on the concentration of the samples, it is reasonable that the enantiomers of 3-hydroxypiperidines obtained from the fractional recrystallization method are optically pure and compare with the results from the enantioselective synthetic pathway.

4. *Convergent Approach*

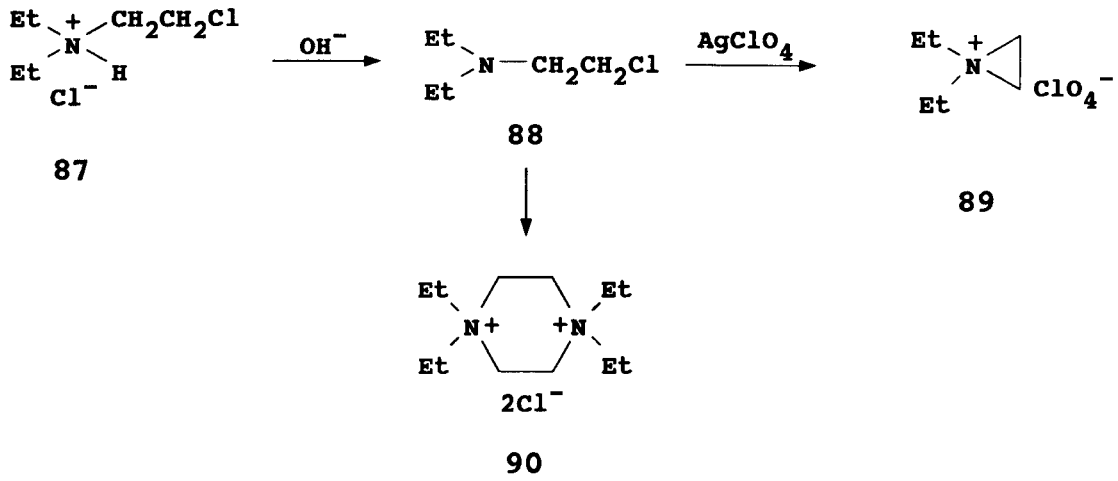
The fractional recrystallization method was very useful for two reasons; to shorten the period of time to aquire material because a large scale process was being contemplated and moreover, fractional recrystallization is relatively inexpensive. In order to access enough of the mustard compounds (**61** - **63** and **78** - **80**) for biological evaluation, the synthesis was resumed after chiral 3-hydroxypiperidines were obtained from fractional recrystallization. However, the neutralization of each diastereomeric salts yielded a poor recovery of 3-hydroxypiperidines (usually less than 60 %), therefore, a solution of Cbz-Cl in toluene was added directly to the solution of salts during free-basing with K_2CO_3 or $NaHCO_3$. After purification by flash column chromatography, the yield of N-Cbz-3-hydroxypiperidines was up to 84.7 %, which means that the recovery of the chiral 3-hydroxypiperidines was higher than 90 %. Acetylation, hydrogenolysis, alkylation, and chlorination were conducted following the procedures described in section A to obtained those mustard compounds (**61** - **63** and **78** - **80**).

C. Formation of a Representative Aziridinium Ion

1. *Making an Ion Pair of Aziridinium Cation with a Mild Weakly Nucleophilic Anion*

The first postulate of the existence of an ethylenimonium or aziridinium compound was given by Marckwald and Frobenius (1901) from the product resulting from treatment of 1-β-chloroethylpiperidine hydrochloride with limited base. It is believed that, considering the reaction condition and the purification method, the product Marckwald and Frobenius obtained was a *bis*-piperidinium salt as more reasonable. Skinner *et al.* (1961) and Hennion and Butler (1962) showed that some of aziridine hydrochloride could be isolated. However, these hydrochloride salts were prepared from relatively stable tertiary amines by addition of ethereal hydrogen chloride. A series of studies of the synthesis of small charged rings have been reported by Leonard's group (1960, 1962, 1963a,b, 1964, 1965a,b). Since the charged three-membered ring was known to open readily in the presence of most anions, including halides, making an ion pair of aziridinium cation with a very weakly nucleophilic anions such as perchlorate and fluoroborate was guided (Leonard, 1962). Bartlett *et al.* (1949) found that β-diethylaminoethyl chloride **88** dimerizes readily in 2:1 acetone-water to give 1,1,4,4-tetraethylpiperazinium dichloride **90** (Scheme XIV). Therefore, anhydrous reaction condition must be used in order to prevent obtaining the dimeric aziridinium salt.

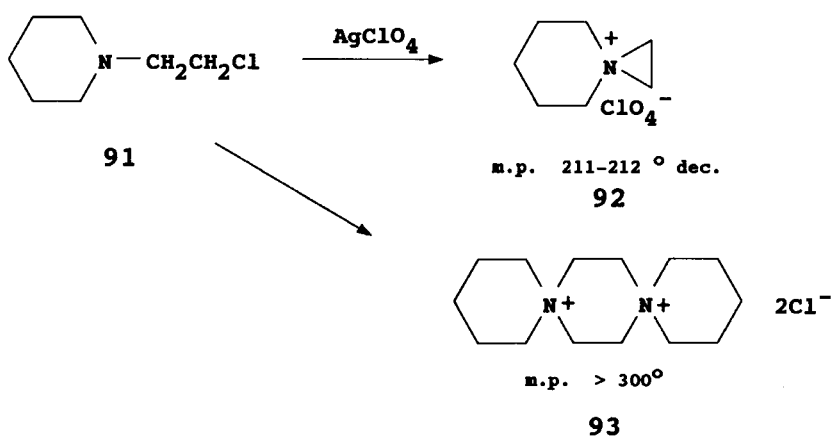
Scheme XIV. Dimerization of β-Diethylaminoethyl Chloride



87 88 89 90

Leonard's group (1965) showed a direct comparison of the "monomeric" and "dimeric" structures of aziridinium salts resulting from N-β-chloroethylpiperidine using NMR and other analytical data (Scheme XV).

Scheme XV. Formation of Monomeric and Dimeric Aziridinium Salts from N-β-Chloroethylpiperidine



91 92 93

2. *Detection of Spirocyclic Aziridinium Cation via NMR*

As illustrated in Scheme XV, the "monomeric" aziridinium, 3-azonia-spiro[2.5]octane perchlorate **92**, shows a great similarity in molecular structure to our target molecules (**31**, **32**, and **33-36**). Therefore, study was continued by treatment of (S)-N-(2-chloroethyl)-3-acetoxypiperidine **61** with silver perchlorate in an anhydrous solvent such as acetone, 2-butanone, or methylene chloride. The former two solvent systems showed much faster progress in reaction than a methylene chloride system. After innumerable trials of purifications, we could obtain (S)-5-acetoxy-3-azoniaspiro[2.5]octane perchlorate **31**, and succeeded in obtaining an ^{1}H NMR spectrum (Fig 19d). The mustard ring protons appear as broad quartets or, possibly, a doublet of quartets at 3.18 ppm. Fig. 18 illustrates the chemical shifts and reasonable multiplicities of the protons of the aziridinium **31**. The low melting point convinced us to believe that we obtained a "monomeric" aziridinium cation.

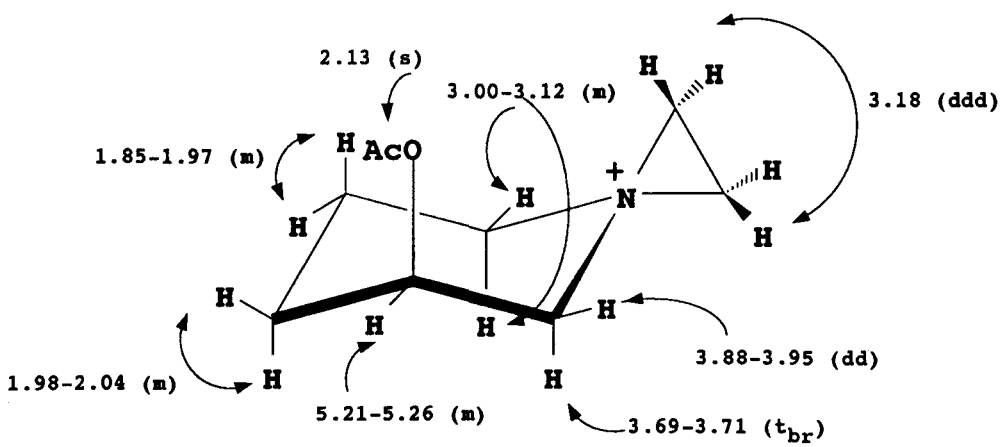


Figure 18. The chemical shifts (ppm) and mutiplicities of the protons of (S)-5-acetoxy-3-azoniaspiro[2.5]octane perchlorate **31**.

Reproduction of this aziridinium was not easy to achieve. However, formation of the aziridinium moiety was also detected by time dependent ^{1}H NMR study (Fig. 19a-d).

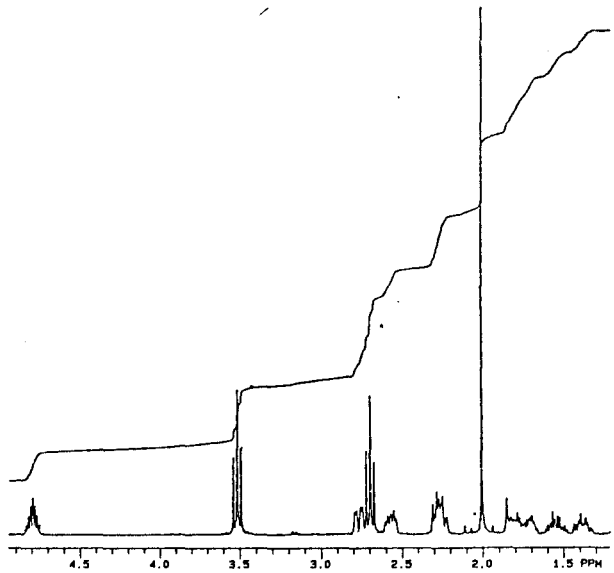


Figure 19a 0 min; ^{1}H NMR of (S)-N-(2-chloroethyl)-3-acetoxypiperidine.

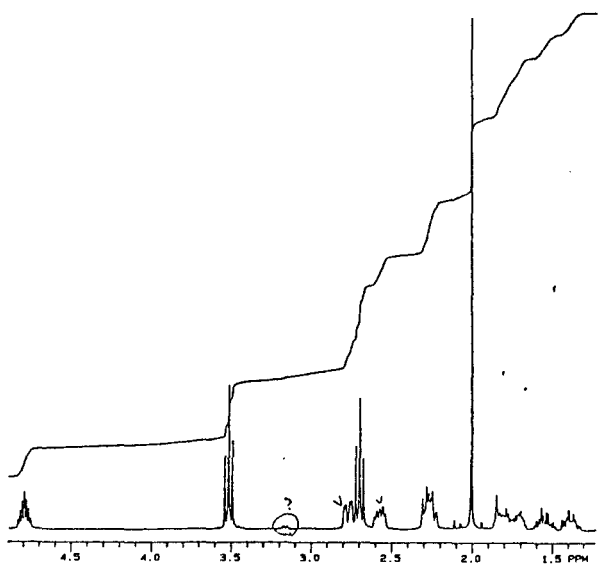


Figure 19b. 15 min after addition of silver perchlorate; The typical quartet peak of the aziridinium cation is about to form (3.10-3.20 ppm). The two triplets from starting material is begun to broaden.

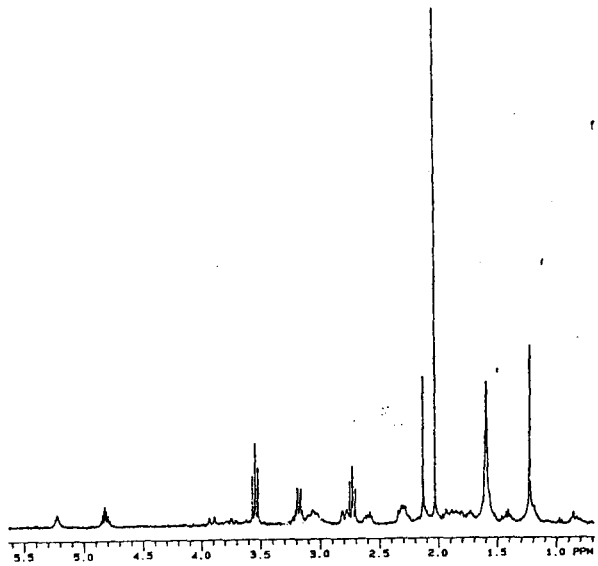


Figure 19c. 1 hour later; the quartet of the ethylenimonium moiety is now shown relatively clearly. The chemical shift of the chiral proton (C_5) is being relocated at further downfield due to the formation of positive charge on the nitrogen.

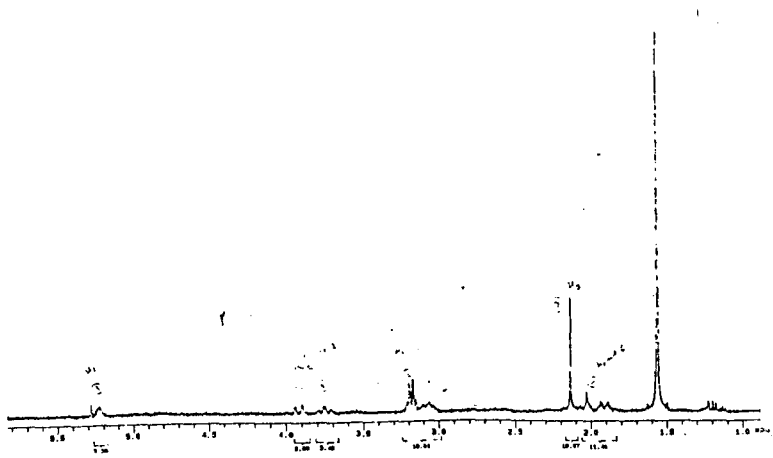


Figure 19d. ^1H NMR of the (S)-5-acetoxy-3-azaniospiro[2.5]octane perchlorate (after purification).

Several other conditions were studied to form the aziridinium moiety. Refluxing the primary chloride precursor compounds (**61** and **78**) in benzene, in the hope of seeing precipitation of aziridinium salt. However, even more than 72 hours of reflux did not show any advance in the reaction. Acetonitrile or DMF solvent systems also did not afford our desired aziridiniums. Triethylamine was added in DMF to generate basic conditions and stirred with neat heating or refluxed, but the same result was observed.

Theoretically, forcing a secondary chloride to undergo a substitution via S_N2 mechanism could be achieved by choosing a solvent system with a highly polar aprotic nature such as DMF, DMSO, or acetone. To avoid sophisticated workup, we first treated the secondary chloro-compounds (**62/63** and **79/80**) with silver perchlorate in anhydrous acetone. However, the crude products were not easy to crystallize. ^1H NMR study indicated that the aziridinium cations, 5-acetoxy-2-methyl-3-azoniaspiro[2.5]octane perchlorates (**33-36**) were formed, but racemization also occurred showing two doublets for the methyl group on the C_2 position.

D. Future work

Enantioselective synthesis of (S)-5-acetoxy-3-azoniaspiro[2.5]octane aziridinium ion **31** has been achieved, and all the reaction conditions and strategies to reach this final stage are optimized. Making the enantiomer of **31** would not be that difficult, however, more study would be required to find better or easier workup method. For the secondary chloro compounds, exploring the proper solvent systems will be the only task. The author believes that these spirocyclic aziridinium ions will show certain level of potency toward the muscarinic receptor, and related biologically active targets. The precursor mustard molecules (chloride compounds) are to be tested for their muscarinic potencies as well. The half-life of this new series of aziridiniums will be studied in the air or in buffer solutions of different pH's.

Finally, as mentioned in Chapter II, development of prodrug delivery systems by attaching a certain length of hydrocarbon chain is to be explored with strategy of modification of carbonyl moieties of the lactams (**42** and **69**).

CHAPTER V

EXPERIMENTAL SECTION

General methods. Commercially available reagents were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, and Fluka Chemical Corp., Ronkonkoma, New York. All solvents and reagents were purified when necessary by standard literature methods (Perrin, 1980). Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was conducted on E. Merck aluminum-backed, 0.2 mm silica gel $60F_{254}$, TLC plates. Visualization was affected with an ultraviolet lamp and/or anisaldehyde stain (2% solution of *o*-anisaldehyde in 95:4:1 absolute ethanol-concentrated sulfuric acid-glacial acetic acid), PMA (phosphomolybdic acid in ethanol), or ninhydrin (5% in ethanol) with heating. Flash chromatography was performed with Kieselgel 60, 230-400 mesh (Merck). Elemental analyses were performed by Midwest Microlab Ltd., Indianapolis, Indiana.

Proton(^{1}H), Carbon (^{13}C) NMR spectra were recorded on a Varian VXR 300-MHz instrument in deuterated chloroform ($CDCl_3$) unless specified otherwise. Pertinent proton frequencies are tabulated in the following order: chemical shift (δ in ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constant (J in Hertz), and the number of

hydrogens. Proton and carbon frequencies of spectra obtained are relative to chloroform (^{1}H, 7.24 ppm; ^{13}C, 77.0 ppm) as an internal standard unless specified otherwise.

Infrared data (IR; $CDCl_3$) were obtained on a Perkin-Elmer Model 1310 instrument. Salient IR features were tabulated in decreasing wavenumber (cm^{-1}). Gas chromatography analyses were performed on a Hewlett-Packard Model 5890 (DB-1 column; range, 50-250 °C; rate, 20 °C/min) and Integrator Model 3392A. Rotation of each molecule was recorded on Perkin-Elmer Model 241 Polarimeter (Na lamp) at room temperature in appropriate solvents (c = 100 x g/mL).

Part I. Enantioselective Synthesis

1. *(S)-(+)-5-Oxo-2-tetrahydrofurancarboxylic acid* **38**

(S)-(+)-glutamic acid **37** (29.9 g, 0.2 mol) was stirred in 4M HCl aq. (130 mL) at ice bath temperature. A solution of $NaNO_2$ (20.7 g, 0.3 mol) in H_2O (45 mL) was added dropwise for a period time of 20 min. Stirring overnight at room temperature resulted in a clear solution and removal of water under reduced pressure yielded white oily solid residue. The residue was shaken with hot acetone (300 mL) and filtered. The insoluble material was washed with additional hot acetone (100 mL). The filtrate and washings were combined. Removal of acetone gave a pale yellow syrup (23.67 g, 91%) which showed similar NMR spectra (for both ^1H and ^{13}C) to the purified product. The last trace of acetone was removed in vacuo, and the viscous residue was solidified after addition of a few seed crystals. The solid was dissolved in portions of hot chloroform (500 mL) and stirred over Na_2SO_4 at ~ 60 °C for 3 hr (most of oil present was captured by Na_2SO_4). The drying agent was filtered and the volume of chloroform solution was reduced to about 200 mL by rotary evaporation. A few seed crystals were added and the solution was chilled at -30 °C. White crystals were obtained (17.7 g, 68.0%) (Mori, 1975; Doolittle and Heath, 1984; Olsen et al., 1985): $[\alpha]_D^{24}$ = +14.8° (c 2.3, CH_3OH); mp 70-72 °C; ^1H NMR δ 2.32-2.42 (m, 1H), 2.52-2.68 (m, 3H), 4.95 (dd, J = 4.2, 8.4 Hz, 1H), 11.48 (s, 1H); ^{13}C NMR δ 25.7, 26.7, 75.2, 174.7, 176.5.

2. *(R)-(-)-5-Oxo-2-tetrahydrofurancarboxylic acid* **65**

Obtained from (R)-(-)-glutamic acid **64**: $[\alpha]_D^{24}$ = -13.8° (c 5.0, CH_3OH); mp 70-72 °C; ^1H NMR δ 2.32-2.42 (m, 1H), 2.52-2.68 (m, 3H), 4.95 (dd. *J* = 4.2, 8.4 Hz, 1H), 11.48 (s, 1H); ^{13}C NMR δ 25.7, 26.7, 75.2, 174.7, 176.5.

3. *(S)-(+)-γ-Hydroxymethyl-γ-butyrolactone* **39**

The lactone carboxylic acid **38** (15.64 g, 0.12 mol) was dissolved in dry THF (80 mL) and stirred for 20 min at -78 °C under argon atmosphere. 2 M borane-methylsulfide in THF (65 mL, 0.13 mol) was injected, warmed up to room temperature, and stirred for 14 hr. The completion of the reaction was monitored by TLC and the mixture was quenched by cautious addition of dry methanol (80 mL). The volatile material and methanol were removed and 50 mL of methanol was added again, stirred, and evaporated under reduced pressure. The residue was purified by flash column chromatography eluting with 4% methanol in chloroform. A colorless liquid **39** was obtained in 91.4% yield (12.75 g) (Yoon et al., 1973; Ravid et al., 1978): TLC (methanol/ chloroform, 4:96), R_f 0.24; $[\alpha]_D^{24}$ = +44.7° (c 0.26, $CHCl_3$); ^1H NMR δ 1.99-2.25 (m, 2H), 2.39-2.60 (m, 2H), 3.51-3.60 (m, 2H), 3.75-3.82 (m, 1H), 4.52-4.59 (m, 1H); ^{13}C NMR δ 23.0, 28.5, 63.8, 80.9, 178.0.

4. *(R)-(-)-γ-Hydroxymethyl-γ-butyrolactone* **66**

This enatiomer was prepared from (R)-(-)-5-oxo-2-tetrahydrofuran-carboxylic acid **65**: TLC (methanol/chloroform, 4:96), R_f 0.24; $[\alpha]_D^{24}$ = -54.4° (c 2.7, $CHCl_3$); ^1H NMR δ 1.98-2.25 (m, 2H), 2.37-2.58 (m, 2H), 3.50-3.59 (m, 2H), 3.77-3.83 (m, 1H), 4.51-4.62 (m, 1H); ^{13}C NMR δ 23.1, 28.5, 63.7, 81.0, 178.4.

5. *(S)-(+)-γ-p-Tosyloxymethyl-γ-butyrolactone* **40**

A mixture of **39** (2,32 g, 20 mmol) and *p*-tosylchloride (5.73 g, 30 mmol) in pyridine (4 mL) and methylene chloride (10 mL) was stirred at room temperature for 9 hr. At completion, the mixture was diluted with methylene chloride (80 mL), and the solution was washed with 10% HCl aq. solution (3 x 50 mL), 20% $NaHCO_3$ aq. solution (2 x 50 mL), and brine. The mixture was dried over Na_2SO_4. The drying agent was filtered and the filtrate was concentrated under reduced pressure. The product was crystallized from methylene chloride/diethyl ether to give the pure tosylate **40** in 93% yield (needle, 5.02 g) (Ho and Davies, 1983): TLC (diethyl ether), R_f 0.16; $[\alpha]_D^{24}$ = +46.2° (c 2.5, $CHCl_3$), mp 86-87 °C; ^1H NMR δ 2.04-2.14 (m, 1H), 2.25-2.37 (m, 1H), 2.42 (s, 3H), 2.46-2.56 (m, 2H), 4.13 (ddd, J = 4.18, 8.69, 11.0 Hz, 2H), 4.62-4.69 (m, 1H), 7.34 (d, J = 8.3 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H); ^{13}C NMR δ 21.6, 23.5, 27.8, 69.9, 76.4, 127.9, 130.0, 132.3, 145.4, 175.9.

6. *(R)-(-)-γ-Tosyloxymethyl-γbutyrolactone* **67**

From (R)-(-)-γ-hydroxymethyl-γ-butyrolactone (**66**): TLC (diethyl ether) R_f 0.16; mp 85-87 °C; $[\alpha]_D^{24}$ = -45.1° (c 2.6, $CHCl_3$); ^1H NMR δ 2.01-2.12 (m, 1H), 2.24-2.35 (m,1H), 2.44 (s, 3H), 2.45-2.55 (m, 2H), 4.12 (ddd, J = 4.19, 8.70, 11.0 Hz, 2H), 4.61-4.68 (m, 1H), 7.34 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H); ^{13}C NMR δ 21.5, 23.5, 27.6, 70.0, 76.5, 127.8, 130.0, 132.3, 145.3, 175.7.

7. *(S)-(+)-5-Azido-4-pentanolide* **41**

To a solution of **40** (8.21 g, 30 mmol) in dry DMF (25 mL) was added NaN_3 (6.5 g, 100 mmol) in portions and refluxed for 1.5 hr. The removal of solvent resulted in an oily residue (dark brown color) which was shaken with chloroform (50 mL) and filtered through a Celite pad. The pad was washed with hot chloroform (20 mL). The filtrate and washing were combined and concentrated *in vacuo*. Flash column chromatography (acetone/hexane, 1:4) afforded yellow liquid product **41** (3.98 g, 93%) (Olsen et al., 1985): TLC (acetone/ hexane, 1:4), R_f 0.09; IR (cm^{-1}) 2120 (N_3 stretching), 1780 (lactone carbonyl); $[\alpha]_D^{24}$ = +91.9° (c 5.0, $CHCl_3$); ^1H NMR δ 1.95-2.08 (m, 1H), 2.23-2.35 (m, 1H), 2.44-2.64 (m, 2H), 3.49 (ddd, J = 3.62, 4.99, 13.3 Hz, 2H), 4.58-4.65 (m, 1H); ^{13}C NMR δ 24.5, 28.1, 54.1, 77.9, 176.0.

8. *(R)-(-)-5-Azido-4-pentanolide* **68**

From (R)-(-)-(**67**), yielded 89.2%: TLC (acetone/hexane, 1:4), R_f 0.10; $[\alpha]_D^{24}$ = -86.3° (c 2.5, $CHCl_3$); ^1H NMR δ 1.95-2.06 (m, 1H), 2.22-2.34 (m, 1H), 2.44-2.65 (m, 2H), 3.47 (ddd, J = 3.62, 5.00, 13.4 Hz, 2H), 4.58-4.66 (m, 1H); ^{13}C NMR δ 24.5, 28.0, 54.2, 78.0, 175.9.

9. *(S)-(-)-5-Hydroxy-2-piperidinone* **42**

(S)-(+)-(**41**) (1.44 g, 10.2 mmol) was dissolved in methanol (80 mL) and catalytic amount of 10% palladium on activated carbon was added. Whole mixture was shaken on a Parr Hydrogenator (H_2, 50 psi) overnight. The solution was filtered through a Celite pad and the pad was washed with hot methanol (20 mL). The filtrate and washing were combined and concentrated under reduced pressure. Flash column chromatography was conducted using methanol/chloroform (1:9) to obtain **42** which was recrystallized from methanol/diethyl ethyl ether to give white crystals (1.15 g, 96%) (Olsen et al., 1985): TLC (methanol/chloroform, 1:9), R_f 0.16; mp 120-121 °C; $[\alpha]_D^{24}$ = -12.4° (c 0.5, CH_3OH); ^1H NMR (D_2O) δ 1.74-1.82 (m, 2H), 2.13-2.36 (m, 2H), 3.13 (dd, J = 3.9, 13.2 Hz, 1H), 3.16 (s_{weak}, 1H), 3.29 (dd, J = 3.8, 13.2 Hz, 1H), 3.53 (d_{br}, 1H), 3.98-4.04 (m, 1H); ^{13}C NMR (D_2O, ref CD_3OD: 49.0 ppm) δ 26.4, 26.6, 47.6, 62.5, 174.9.

10. *(R)-(+)-5-Hydroxy-2-piperidinone* **69**

From (R)-(-)-(**68**), yield (92%): mp 120-123 °C; $[\alpha]_D^{24}$ = +13.3° (c 0.5, CH_3OH); ^1H NMR δ 1.73-1.88 (m, 2H), 2.15-2.38 (m, 2H), 3.09 (dd, *J* = 3.9, 13.2 Hz, 1H), 3.19 (s_{weak}, 1H), 3.31 (dd, *J* = 3.8, 13.2 Hz, 1H), 3.52 ($d_{br,weak}$, 1H), 4.00-4.05 (m, 1H); ^{13}C NMR δ 26.3, 26.5. 47.8, 62.7, 175.1.

11. *(S)-(-)-3-Hydroxypiperidine* **43**

Method A. (S)-(-)-5-Hydroxy-2-piperidinone **42** (970 mg, 8.43 mmol) was stirred in 20 mL of dry THF at 0 °C under argon atmosphere. $LiAlH_4$ (640 mg, 2 eq) was added in portions and the ice bath was removed. The whole reaction mixture was refluxed for 43.5 hr. The reaction was diluted with ethyl acetate (50 mL) and a minimum amount of 0.1 M NaOH aq. solution was added to quench the excess reagent left over. The insoluble material was filtered and dried over Na_2SO_4. The drying agent was filtered and removal of solvent gave 1.4 g of residue which was purified by flash column chromatography (methanol/chloroform, 3:7) to yield **43** (pale yellow crystal, 434 mg, 59.6%).

Method B. To a suspension of the lactam **42** in dry THF (33.3 mg/mL), 1M BH_3•THF complex in THF was injected at 0°C under argon atmosphere. The ice bath was removed and reaction mixture was refluxed for 1.5 hr. The reaction was quenched by cautious addition of anhydrous methanol and concentrated under reduced pressure. Same condition of column chromatography was conducted to yield **43** (48-62%): TLC (methanol

/chloroform, 3:7), R_f 0.01; mp 55-60 °C; $[\alpha]_D^{24}$ = -8.94° (c 4.7, CH_3OH); ^1H NMR δ 1.37-1.55 (m, 2H), 1.68-1.82 (m, 2H), 2.17 (s_{br}, 2H), 2.61 (dd, J = 6.8, 11.9 Hz, 1H), 2.63-2.76 (m, 2H), 2.94 (dd, J = 2.5, 11.9 Hz, 1H), 3.63-3.69 (m, 1H); ^{13}C NMR δ 23.4, 32.9, 46.4, 53.5, 66.6.

12. *(R)-(+)-3-Hydroxypiperidine* **70**

From (R)-(+)-(**69**): yield 58.3%; TLC (methanol/chloroform, 3:7), R_f 0.01; mp 56-60 °C; $[\alpha]_D^{24}$ = +9.4° (c 0.65, CH_3OH); ^1H NMR δ 1.38-1.59 (m, 2H), 1.70-1.83 (m, 2H), 2.15 (s_{br}, 2H), 2.63 (dd, J = 6.8, 12.0 Hz, 1H), 2.65-2.77 (m, 2H), 2.93 (dd, J = 2.5, 12.0 Hz, 1H), 3.65-3.70 (m, 1H); ^{13}C NMR δ 23.6, 32.9, 46.2, 53.5, 66.4.

13. *(S)-(+)-N-Cbz-3-Hydroxypiperidine* **54**

Method A. (S)-(-)-3-Hydroxypiperidine **43** (1.012 g, 10 mmol) was stirred in 10 mL of water with 2 equivalent mole of powdered sodium bicarbonate (1.68 g). A solution of carbobenzyloxy chloride (2.85 mL, 20 mmol) in toluene (8 mL) was added dropwise and pH was controlled to 8~9 by addition of 50% NaOH aq. solution. The solution was stirred overnight at room temperature. Diethyl ether (80 mL) was added and washed with water and sat'd NaCl aq. solution. Following treatment with Na_2SO_4, the drying agent was filtered, and concentrated by rotary evaporator. Flash column chromatography was conducted using petroleum ether/diethyl ether (2:3) to obtain a colorless oil (**54**)

(2.23 g, 88%). With commercially available racemic mixture (Fluka), this reaction completed in higher yield (90-95%).

Method B. (from lactam **42**). The (S)-(-)-lactam crystals (**42**) (415 mg, 3.6 mmol) was stirred in dry THF (15 mL) at 0 °C under argon atmosphere. After a few minutes, 1 M BH_3•THF complex in THF (15 mL, 15 mmol) was injected slowly and the solution was heated (~ 60 °C) for 1.5 hr. The reaction was quenched by cautious addition of methanol and removal of volatile material and solvent under reduced pressure gave yellowish oily crystals (piperidinol). The crude 3-hydroxypiperidine was dissolved in 7 mL of water, without further purification, and sodium bicarbonate (0.8 g) was added. Few minutes later, a solution of carbobenzyloxy chloride (1.4 mL) in toluene (5 mL) was added dropwise. After adjusting pH 8~9 with 50% NaOH aq. solution, the reaction was stirred overnight at room temperature. The work up process is same as described in method A, and 642 mg of **54** was yielded (overall yield 75.8%): TLC (petroleum ether/diethyl ether, 2:3), R_f 0.18; t_R 7.29 min; $[\alpha]_D^{24}$ = +5.79° (c 3.8, $CHCl_3$); ^1H NMR δ 1.38-1.59 (m, 2H), 1.71-1.93 (m, 2H), 3.08-3.28 (m, 2H), 3.51-3.67 (br, 1H), 3.68-3.78 (br, 1H), 3.76 (s, 1H), 3.80-3.83 (m, 1H), 5.23 (s, 2H), 7.26-7.38 (m, 5H); ^{13}C NMR δ 22.2, 32.3, 44.1, 50.7, 66.0, 67.2, 126.9, 128.0, 136.7, 155.7.

14. *(R)-(-)-N-Cbz-3-Hydroxypiperidine* **71**

From either (R)-(+)-(**69**) or (R)-(+)-(**70**), yield 85.4% (white oily crystal): TLC (petroleum ether/diethyl ether), R_f 0.16; t_R 7.28 min; $[\alpha]_D^{24}$ = -6.44° (c 2.64, $CHCl_3$); ^1H NMR δ 1.39-1.59 (m, 2H), 1.71-1.92 (m, 2H), 3.09-3.28 (m, 2H), 3.51-3.66 (br, 1H), 3.68-3.79 (br, 1H), 3.75 (s, 1H), 3.80-3.84 (m, 1H), 5.23 (s, 2H), 7.26-7.38 (m, 5H); ^{13}C NMR δ 22.2, 32.1, 44.0, 50.8, 66.1, 67.3, 126.8, 128.1, 136.7, 155.7

15. *(S)-(-)-N-Cbz-3-Acetoxypiperidine* **55**

The colorless oil **54** (1.12 g, 4.76 mmol) was dissolved in 1 mL of pyridine and catalytic amount of DMAP (N,N-dimethylaminopyridine) was added. The mixture was stirred at room temperature under argon atmosphere. Anhydrous acetic anhydride (0.6 mL, 6.4 mmol) was injected and stirred for 3 hr. Ethyl acetate (40 mL) was added and washed with 20% $NaHCO_3$ and sat'd NaCl aq. solution. After dried over Na_2SO_4, filtered, and solvent was removed by rotary evaporator. The residue was purified by flash column chromatography using petroleum ether/diethyl ether (1:1) to yield 1.12 g (84.8%) of colorless liquid **55**: TLC (petroleum ether/diethyl ether, 1:1), R_f 0.21; t_R 10.10 min; $[\alpha]_D^{24}$ = -10.5° (c 4.0, $CHCl_3$); ^1H NMR δ 1.43-1.60 (m, 1H), 1.63-1.90 (m, 3H), 1.98 (s_{br}, 3H), 3.29-3.43 (m, 1H), 3.45-3.67 (m, 3H), 4.72-4.86 (m, 1H), 5.13 (s_{br}, 2H), 7.25-7.38 (m,5H); ^{13}C NMR δ 20.9, 21.8, 28.9, 44.0, 47.3, 67.0, 67.7, 127.7, 127.9, 128.4, 136.7, 155.4, 170.2. Anal. calcd for $C_{15}H_{19}NO_4$

: C, 64.97; H, 6.91; N, 5.05. Found: C, 64.93; H, 6.91; N, 5.10.

16. *(R)-(+)-N-Cbz-3-Acetoxypiperidine* **72**

From (R)-(-)-(**54**), yielded 82.6%: TLC (petroleum ether/diethyl ether, 1:1), R_f 0.20; t_R 10.09 min; $[\alpha]_D^{24}$ = +11.2° (c 4.3, $CHCl_3$); ^1H NMR δ 1.43-1.60 (m, 1H), 1.63-1.90 (m, 3H), 1.98 (s_{br}, 3H), 3.29-3.43 (m, 1H), 3.45-3.67 (m, 3H), 4.72-4.86 (m, 1H), 5.13 (s_{br}, 2H), 7.25-7.38 (m,5H); ^{13}C NMR δ 20.9, 21.8, 28.9, 44.0, 47.3, 67.0, 67.7, 127.7, 127.9, 128.4, 136.7, 155.4, 170.2.

17. *(S)-(-)-3-Acetoxypiperidine* **51**

To a solution of **55** (1.01 g, 3.64 mmol) in methanol (80 mL) was added a catalytic amount of 10% palladium on activated carbon. The mixture was shaken under 35 psi of hydrogen on a Parr Hydrogenator. The catalyst was filtered through a Celite pad and the pad was washed with 10 mL of methanol. The filtrate and washing were combined and concentrated under reduced pressure. Flash column chromatography was conducted with chloroform/ methanol (9:1) to give colorless liquid **51** (468 mg, 89.8%): TLC (chloroform /methanol, 9:1), R_f 0.10; t_R 4.49 min; $[\alpha]_D^{24}$ = -8.43° (c 7.0, $CHCl_3$); ^1H NMR δ 1.37-1.55 (m, 2H), 1.55-1.78 (m, 2H), 1.82-1.93 (m, 1H), 2.01 (s, 3H), 2.63-2.75 (m, 3H), 2.96-3.08 (d_{br}, 1H), 4.67-4.76 (m, 1H); ^{13}C NMR δ 21.3, 24.2, 29.6, 45.9, 50.1, 69.6, 170.5. Anal. Calcd for $C_7H_{13}NO_2$: C, 58.67; H, 9.15; N, 9.78. Found: C, 58.81; H, 9.16; N, 9.64.

18. *(R)-(+)-3-Acetoxypiperidine* **73**

From (R)-(+)-(**72**), yield (85.4%): TLC (chloroform/methanol, 9:1), R_f 0.10; t_R 4.48 min; $[\alpha]_D^{24}$ = +9.14° (c 5.8, $CHCl_3$); ^1H NMR δ 1.35-1.54 (m, 2H), 1.55-1.80 (m, 2H), 1.83-1.95 (m, 1H), 2.02 (s, 3H), 2.65-2.76 (m, 3H), 2.95-3.06 (d_{br}, 1H), 4.67-4.77 (m, 1H); ^{13}C NMR δ 21.2, 24.1, 29.5, 46.0, 50.0, 69.4, 170.6.

19. *(S)-(-)-N-(2-Hydroxyethyl)-3-acetoxypiperidine* **58**

The colorless liquid **51** (400 mg, 2.79 mmol) was stirred in methanol (20 mL) at ice-bath temperature. Few minutes later, ethylene oxide was bubbled into the solution and the proceeding of the reaction was monitored by TLC. Reaction was stopped when the formation of the overalkylated quaternary ammonium was monitored and the excess ethylene oxide was degassed, and the solution was concentrated *in vacuo*. Flash column chromatography using methanol/chloroform (2:98) afforded colorless liquid **58** (334 mg, 63.9%): TLC (methanol/chloroform, 1:9), R_f 0.29; t_R 6.49 min; $[\alpha]_D^{24}$ = -24.0° (c 0.9, $CHCl_3$), ^1H NMR δ 1.40-1.57 (m, 2H), 1.70-1.83 (m, 2H), 2.00 (s, 3H), 2.20-2.30 (m, 2H), 2.47-2.51 (t, J = 5.9 Hz, 2H), 2.53-2.58 (m, 1H), 2.72-2.77 (dd, J = 3.4, 11.0 Hz, 2H), 3.53-3.56 (t, J = 5.9 Hz, 2H), 4.76-4.84 (m, 1H); ^{13}C NMR δ 21.2, 22.7, 29.3, 53.0, 56.9, 57.7, 59.1, 69.4, 170.4. Anal calcd for $C_9H_{17}NO_3$: C, 57.73; H, 9.15; N, 7.48. Found: C, 57.62; H, 9.11; N, 7.19.

20. *(R)-(+)-N-(2-Hydroxyethyl)-3-acetoxypiperidine* **75**

From (R)-(+)-3-acetoxypiperidine **73**, yielded 58.8%: TLC (methanol / chloroform, 1:9), R_f 0.30; t_R 6.48 min; $[\alpha]_D^{24}$ = +22.1° (c 1.1, $CHCl_3$); ^1H NMR δ 1.41-1.58 (m, 2H), 1.70-1.83 (m, 2H), 1.99 (s, 3H), 2.20-2.29 (m, 2H), 2.49 (t, *J* = 5.9 Hz, 2H), 2.53-2.58 (m, 1H), 2.75 (dd, *J* = 3.4, 11.0 Hz, 2H), 3.55 (t, *J* = 5.9 Hz, 2H), 4.76-4.84 (m, 1H); ^{13}C NMR δ 21.2, 22.8, 29.3, 52.9, 57.0, 57.7, 59.1, 69.4, 170.5.

21. *(S,S)-(+)-N-(2-Hydroxypropyl)-3-acetoxypiperidine* **59**

(S)-(-)-3-acetoxypiperidine **51** (578 mg, 4.0 mmol) was stirred in methanol (5 mL) at 5°C. (S)-(-)-propylene oxide (0.4 mL, 5.7 mmol) was added dropwise and the reaction was stirred for 14-17 hr at 5 °C. At completion, the volatile material and methanol were removed under reduced pressure, then the residue was purified by flash column chromatography using diethyl ether as eluting system to yield (S,S)-(+)-(**59**) (612 mg, 76%). TLC (diethyl ether), R_f 0.25; t_R 6.65 min; $[\alpha]_D^{24}$ = +24.4° (c 3.6, $CHCl_3$); ^1H NMR δ 1.07 (d, *J* = 6.2 Hz, 3H), 1.39-1.60 (m, 2H), 1.67-1.84 (m, 2H), 1.99 (s, 3H), 2.11-2.22 (m, 2H), 2.28 (dd, *J* = 3.1, 12.5 Hz, 1H), 2.42 (t_{br}, *J* = 5.3 Hz, 2H), 2.86 (dd, *J* = 3.2, 11.0 Hz, 1H), 3.72-3.80 (m, 1H), 4.78 (t on dd, *J* = 7.8, 7.9, 15.7 Hz, 1H); ^{13}C NMR δ 19.9, 21.2, 22.7, 29.2, 52.8, 57.5, 62.2, 65.5, 69.2, 170.4. Anal calcd for $C_{10}H_{19}NO_3$: C, 59.68; H, 9.52; N, 6.96. Found: C, 59.63, H, 9.54, N, 6.92.

22. *(R,R)-(-)-N-(2-Hydroxypropyl)-3-acetoxypiperidine* **77**

This enantiomer of (S,S)-(+)-(**59**) was obtained from (R)-(+)-3-acetoxypiperidine **73** and (R)-(+)-propylene oxide (68% yield): TLC (diethyl ether), R_f 0.25; t_R 6.64 min; $[\alpha]_D^{24}$ = -22.6° (c 3.4, $CHCl_3$); ^{1}H NMR δ 1.07 (d, J = 6.2 Hz, 3H), 1.40-1.61 (m, 2H), 1.68-1.85 (m, 2H), 2.00 (s, 3H), 2.11-2.23 (m, 2H), 2.27 (dd, J = 3.2, 12.5 Hz, 1H), 2.42 (t_{br}, J = 5.2 Hz, 2H), 2.86 (dd, J = 3.2 Hz, 11.2 Hz, 1H), 3.71-3.79 (m, 1H), 4.79 (t on dd, J = 7.8, 8.0, 15.6 Hz, 1H); ^{13}C NMR δ 20.0, 21.2, 22.6, 29.1, 53.0, 57.5, 62.3, 65.6, 69.4, 170.5.

23. *(S,R)-(+)-N-(2-Hydroxypropyl)-3-acetoxypiperidine* **76**

This diastereoisomer of (S,S)-(+)-(**59**) was obtained from the reaction of (R)-(+)-3-acetoxypiperidine **73** and (S)-(-)-propylene oxide (yield 71%): TLC (diethyl ether), R_f 0.19; t_R 6.64 min; $[\alpha]_D^{24}$ = +73.3° (c 3.6, $CHCl_3$); ^{1}H NMR δ 1.08 (d, J = 8.3 Hz, 3H), 1.41-1.56 (m, 2H), 1.72-1.84 (m, 2H), 2.00 (s, 3H), 2.10-2.19 (m, 2H), 2.31 (dd, J = 3.1, 12.3 Hz, 1H), 2.44 (t_{br}, J = 7.6 Hz, 1H), 2.62-2.72 (m, 2H), 3.73-3.79 (m, 1H), 4.82 (t on dd, J = 7.7, 7.8, 15.3 Hz, 1H); ^{13}C NMR δ 19.9, 21.2, 22.7, 29.2, 52.8, 57.5, 62.2, 65.5, 69.2, 170.4.

24. *(R,S)-(-)-N-(2-Hydroxypropyl)-3-acetoxypiperidine* **60**

From (S)-(-)-3-acetoxypiperidine **51** and (R)-(+)-propylene oxide (yield 78%): TLC (diethyl ether), R_f 0.14; t_R 6.55 min; $[\alpha]_D^{24}$ = -68.9° (c 6.3, $CHCl_3$); ^{1}H NMR δ 1.10 (d, J = 8.2 Hz, 3H), 1.42-1.56 (m, 2H), 1.72-1.84 (m, 2H), 2.01

(s, 3H), 2.10-2.20 (m, 2H), 2.31 (dd, J = 3.2, 12.4 Hz, 1H), 2.45 (t_{br}, J = 7.6 Hz, 1H), 2.63-2.72 (m, 2H), 3.74-3.81 (m, 1H), 4.82 (t on dd, J = 7.6, 7.8, 15.4 Hz, 1H); ^{13}C NMR δ 20.0, 21.2, 22.6, 29.4, 52.6, 57.6, 62.2, 65.6, 69.3, 170.3.

25. *(S)-(-)-N-(2-Chloroethyl)-3-acetoxypiperidine* **61**

(S)-(-)-(**58**) (373 mg, 2.0 mmol) was stirred in dry methylene chloride (6 mL) at 5 °C under argon atmosphere. Freshly distilled thionyl chloride (0.16 mL, 2.2 mmol) was injected and stirred for 20 min. The cooler was removed and the reaction was allowed to stir for more 3 hr at room temperature. At completion, the reaction was diluted by addition of methylene chloride (10 mL), and chilled at ice bath temperature. Addition of dry diethyl ether resulted in crystallization of hydrochloride salt. The salt was filtered and washed with dry diethyl ether. Removal of the last trace of the solvent in vacuo gave white crystals of (S)-(-)-(**61**) hydrochloride salt (408 mg, 80.4%). The (S)-(-)-(**61**) hydrochloride salt was redissolved in methylene chloride (10 mL) and excess amount of sodium bicarbonate was added. Vigorous stirring resulted in free base (tertiary amine) which was purified by flash column chromatography using petroleum ether and diethyl ether (1:1): TLC (petroleum ether/diethyl ether, 1:1), R_f 0.34; $[\alpha]_D^{24}$ = -31.1° (c 0.45, $CHCl_3$); ^1H NMR δ 1,38-1.46 (m, 1H), 1.52-1.62 (m, 1H), 1.71-1.87 (m, 2H), 2.03 (s, 3H), 2.25-2.34 (m, 2H), 2.56-2.63 (m, 1H), 2.73 (t, J = 7.2 Hz, 2H), 2.80 (dd_{br}, J = 3.55, 10.9, Hz, 1H), 3.54 (t, J = 7.2 Hz, 2H), 4.82 (t on dd, J = 7.8, 8.2, 15.9 Hz, 1H); ^{13}C NMR δ 21.3,

22.6, 29.2, 40.8, 53.3, 56.9, 59.7, 69.3, 170.5. Anal calcd for $C_9H_{16}NO_2Cl$: C, 52.55; H, 7.84; N, 6.81. Found: C, 52.55; H, 7.86; N, 6.74.

26. *(R)-(+)-N-(2-Chloroethyl)-3-acetoxypiperidine* **78**

From (R)-(+)-(**75**): R_f 0.35; $[\alpha]_D^{24}$ = +28.3° (c 0.50, $CHCl_3$); ^1H NMR δ 1.38-1.46 (m, 1H), 1.51-1.63 (m, 1H), 1.72-1.88 (m, 2H), 2.02 (s, 3H), 2.26-2.33 (m, 2H), 2.55-2.62 (m, 1H), 2.73 (t, J = 7.2 Hz, 2H), 4.80 (t on dd, J = 7.8, 8.2, 16.0 Hz, 1H); ^{13}C NMR δ 21.4, 22.6, 29.6, 41.0, 53.1, 57.1, 59.9, 69.5, 170.4.

Reaction of N-(2-hydroxypropyl)-3-acetoxypiperidine with thionyl chloride

General procedure; Each diastereoisomer of N-(2-hydroxypropyl)-3-acetoxypiperidine was stirred in dry methylene chloride (50 mg / mL) at 0 °C under argon atmosphere. Few minutes later freshly distilled thionyl chloride (1.5 equiv) was injected and the reaction mixture was stirred for 20 min. The cooler was removed and the reaction was allowed to stir 5 more hr at room temperature. With stirring, addition of dry diethyl ether caused crystallization of hydrochloride salt. Filtered and recrystallization from methylene chloride/diethyl ether afforded white crystals (71-84%). Treatment with base and a flash column chromatography using petroleum ether and diethyl ether (3:1) as eluting system afforded tertiary amines **62**, **63**, **79**, and **80**. Anal calcd for $C_{10}H_{18}NO_2Cl$: C, 54.66; H, 8.26; N, 6.38. Found: C, 55.00; H, 8.10; N, 6.21.

27. *(S,S)-(-)-N-(2-Chloropropyl)-3-acetoxypiperidine* **62**

mp 134-137 °C (HCl salt); TLC (petroleum ether/diethyl ether, 3:1), R_f 0.32; $[\alpha]_D^{24}$ = -43.0 (c 2.6, $CHCl_3$); ^1H NMR δ 1.29-1.41 (m, 1H), 1.47 (d, J = 6.4 Hz, 3H), 1.51-1.62 (m, 1H), 1.66-1.77 (m, 1H), 1.79-1.89 (m, 1H), 2.01 (s, 3H), 2.19-2.29 (m, 2H), 2.46 (dd, J = 7.4, 13.1 Hz, 1H), 2.55-2.61 (m, 1H), 2.63 (dd, J = 6.4, 13.1 Hz, 1H), 2.80 (dd_{br}, J = 3.9, 10.8 Hz, 1H), 4.01 (dq, J = 6.7, 13.2 Hz, 1H), 4.78 (t on dd, J = 8.3, 8.6, 16.8 Hz, 1H); ^{13}C NMR δ 21.2, 22.9, 23.2, 29.4, 53.8, 54.6, 57.2, 66.1, 69.5, 170.4.

28. *(R,R)-(+)-N-(2-Chloropropyl)-3-acetoxypiperidine* **79**

mp 133-137 °C (HCl salt); TLC (petroleum ether/diethyl ether, 3:1), R_f 0.31; $[\alpha]_D^{24}$ = +30.0° (c 1.8, $CHCl_3$); ^1H NMR δ 1.31-1.41 (m, 2H), 1.47 (d, J = 6.54 Hz, 3H), 1.51-1.63 (m, 1H), 1.68-1.75 (m, 1H), 1.79-1.87 (m, 1H), 2.01 (s, 3H), 2.20-2.30 (m, 2H), 2.47 (dd, J = 7.3, 13.1 Hz, 1H), 2.55-2.64 (m, 1H), 2.64 (dd, J = 6.4, 13.1 Hz, 1H), 2.78-2.83 (m, 1H), 3.96-4.05 (m, 1H), 4.78 (t on dd, J = 8.2, 8.5, 16.7 Hz, 1H); ^{13}C NMR δ 21.3, 22.8, 23.2, 29.4, 53.8, 54.6, 57.2, 66.1, 69.5, 170.4.

29. *(S,R)-(-)-N-(2-Chloropropyl)-3-acetoxypiperidine* **80**

mp 153-155 °C (HCl salt); TLC (petroleum ether/diethyl ether, 3:1), R_f 0.33; $[\alpha]_D^{24}$ = -21.1° (c 0.9, CH_3OH) (HCl salt); ^1H NMR δ 1.31-1.41 (m, 2H), 1.47 (d, J = 6.54 Hz, 3H), 1.51-1.63 (m, 1H), 1.68-1.75 (m, 1H), 1.79-1.87 (m,

1H), 2.01 (s, 3H), 2.20-2.30 (m, 2H), 2.47 (dd, J = 7.3, 13.1 Hz, 1H), 2.55-2.64 (m, 1H), 2.64 (dd, J = 6.4, 13.1 Hz, 1H), 2.78-2.83 (m, 1H), 3.96-4.05 (m, 1H), 4.78 (t on dd, J = 8.2, 8.5, 16.7 Hz, 1H); ^{13}C NMR δ 21.3, 22.8, 23.2, 29.4, 53.8, 54.6, 57.2, 66.1, 69.5, 170.4.

30. *(R,S)-(+)-N-(2-Chloropropyl)-3-acetoxypiperidine* **63**

mp 152-154 °C (HCl salt); $[\alpha]_D^{24}$ = +24.2° (c 1.2, CH_3OH) (HCl salt); $_1$H NMR δ 1.31-1.41 (m, 2H), 1.47 (d, J = 6.54 Hz, 3H), 1.51-1.63 (m, 1H), 1.68-1.75 (m, 1H), 1.79-1.87 (m, 1H), 2.01 (s, 3H), 2.20-2.30 (m, 2H), 2.47 (dd, J = 7.3, 13.1 Hz, 1H), 2.55-2.64 (m, 1H), 2.64 (dd, J = 6.4, 13.1 Hz, 1H), 2.78-2.83 (m, 1H), 3.96-4.05 (m, 1H), 4.78 (t on dd, J = 8.2, 8.5, 16.7 Hz, 1H); ^{13}C NMR δ 21.3, 22.8, 23.2, 29.4, 53.8, 54.6, 57.2, 66.1, 69.5, 170.4.

31. *(S)-(-)-N,N-Dimethyl-3-acetoxypiperidinium iodide* **21**

To a solution of (S)-(-)-3-acetoxypiperidine **51** (430 mg, 3.0 mmol) in anhydrous methanol (3 mL), methyl iodide (1 mL, excess) was added dropwise at room temperature. At completion, the reaction mixture was diluted with methanol (12 mL). With stirring, addition of anhydrous diethyl ether afforded white powdery crystals of **21** in 65% yield: mp 165-168 °C; $[\alpha]_D^{24}$ = -3.36° (c 0.4, $CHCl_3$); ^{1}H NMR δ 1.90-2.05 (m, 2H), 2.10 (s, 3H), 2.07-2.22 (m, 2H), 3.50 (s, 3H), 3.55 (s, 3H), 3.71-3.83 (m, 2H), 3.97-4.08 (m, 1H), 4.16 (dd, J = 3.3, 13.5 Hz, 1H), 5.22-5.28 (m, 1H); ^{13}C NMR δ 17.13, 21.24, 25.33, 53.13, 55.40,

62.19, 63.44, 65.28, 169.31.

32. *(R)-(+)-N,N-Dimethyl-3-acetoxypiperidinium iodide* **74**

From (R)-(+)-3-acetoxypiperidine **73**, yield 61%: mp 166-169 °C; $[\alpha]_D^{24}$ = +3.82° (c 0.5, $CHCl_3$); ^1H NMR δ 1.90-2.04 (m, 2H), 2.09 (s, 3H), 2.07-2.21 (m, 2H), 3.50 (s, 3H), 3.56 (s, 3H), 3.70-3.82 (m, 2H), 4.01-4.09 (m, 1H), 4.13 (dd, *J* = 3.4, 13.5 Hz, 1H), 5.20-5.25 (m, 1H); ^{13}C NMR δ 17.14, 21.24, 25.34, 53.14, 55.40, 62.18, 63.45, 65.30, 169.33.

Part II. Fractional Recrystallization

33. *(R,R)-(+)-Diacetoxysuccinic anhydride* **83**

(+)-Tartaric acid (15.09 g, 0.1 mol) was placed in 250 mL round bottom flask and connected to a condenser. The condenser was attached to an additional funnel and covered with an argon balloon. A solution of acetic anhydride (40 mL) and sulfuric acid (1.0 mL) was added dropwise with stirring at 0°C. At completion of addition, the cooler was removed and the mixture was refluxed for 30 min. Cooling at room temperature resulted in a solid residue. The solidified residue was shaken with benzene (100 mL) and filtered. The crystals were washed with dry ethyl ether. Removal of the last trace of solvent under vacuum afforded white crystals **83** in 76% yield: mp 133-135 °C (dec.) (lit. 133-134 °C); $[\alpha]_D^{24}$ = +97.2° (c, 0.5, $CHCl_3$); ^1H NMR δ 2.21 (s, 6H), 5.66 (s, 2H); ^{13}C NMR δ 20.16, 22.19, 72.07, 163.09, 169.50.

34. *(S,S)-(-)-Diacetoxysuccinic anhydride* **84**

This enantiomer of **83** was prepared from (-)-tartaric acid: mp 133-134 °C; $[\alpha]_D^{24}$ = -98.5° (c, 0.5, $CHCl_3$); ^1H NMR δ 2.22 (s, 6H), 5.67 (s, 2H); ^{13}C NMR δ 20.15, 22.19, 72.08, 163.09, 169.51.

35. *(R,R)-(+)-4-Chlortartranilic acid* **85**

To a solution of **83** (10 g, 46.3 mmol) in dry methylene chloride (50 mL), 4-chloroaniline (6.0 g, 47 mmol) was added portionwise and whole solution was

refluxed for 15 hr. After cooling, the dark brown solution was washed with 3 equivalents of potassium hydroxide aqueous solution and the washings were combined and neutrallized by addition of conc. HCl resulting precipitation of crude product. The wet crystals were recrystallized from ethanol/water to obtain needle-shaped colorless crystals **85** in 68% yield: mp 192-194 °C (dec.); ^{1}H NMR δ 3.50-4.20 (br, 2H), 4.57 (s, 1H), 4.67 (s, 1H), 5.11-5.40 (br, 1H), 7.34 (d, *J* = Hz, 2H), 7.80 (d, *J* = Hz, 2H), 9.32 (s, 1H); ^{13}C NMR δ 72.67, 74.25, 121,75, 129.31, 170.45, 173.44.

36. *(S,S)-(-)-4-Chlorotartranilic acid* **86**

Prepared from **84**: mp 193-195 °C (dec.); ^{1}H NMR δ 3.50-4.21 (br, 2H), 4.58 (s, 1H), 4.66 (s, 1H), 5.10-5.39 (br, 1H), 7.34 (d, *J* = Hz, 2H), 7.81 (d, *J* = Hz, 2H), 9.35 (s, 1H); ^{13}C NMR δ 72.66, 74.25, 121.72, 129.27, 170.44, 173.46.

37. *Fractional recrystallization of the diastereomeric salts and recovery of optically pure 3-piperidine from its diastereomeric salt.*

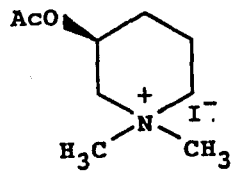
(+)-(+)-salt: To a solution of (+/-)-3-hydroxypiperidine in warm 95% ehtanol, a solution of 0.5 equivalent of (+)-4-chlorotartranilic acid **85** in 95% ethanol was added. Stirred at 40 °C to obtain clear solution state, then cooled to room temperature. Standing overnight at room temperature yielded needle-shaped crystals in 70-74% yield: mp 153-156 °C.

(-)-(-)-salt: Obtained from (-)-4-chlorotartranilic acid **86**: mp 152-156 °C.

Each diastereomeric salt was dissolved in methanol/toluene (3:7) and equivalent amount of potassium carbonate was added. After vigorous stirring for 3 hr, filtered, and the filtrate was concentrated in vacuo to give a waxy solid residue. The residue was shaken with hot ethyl acetate and concentrated. Crystallization from ethyl acetate/benzene/hexane gave pale yellow crystals (powdery) of (S)-(-)- or (R)-(+)-3-hydroxypiperidine in 56-62% of recovery.

APPENDIX

SELECTED SPECTRAL DATA



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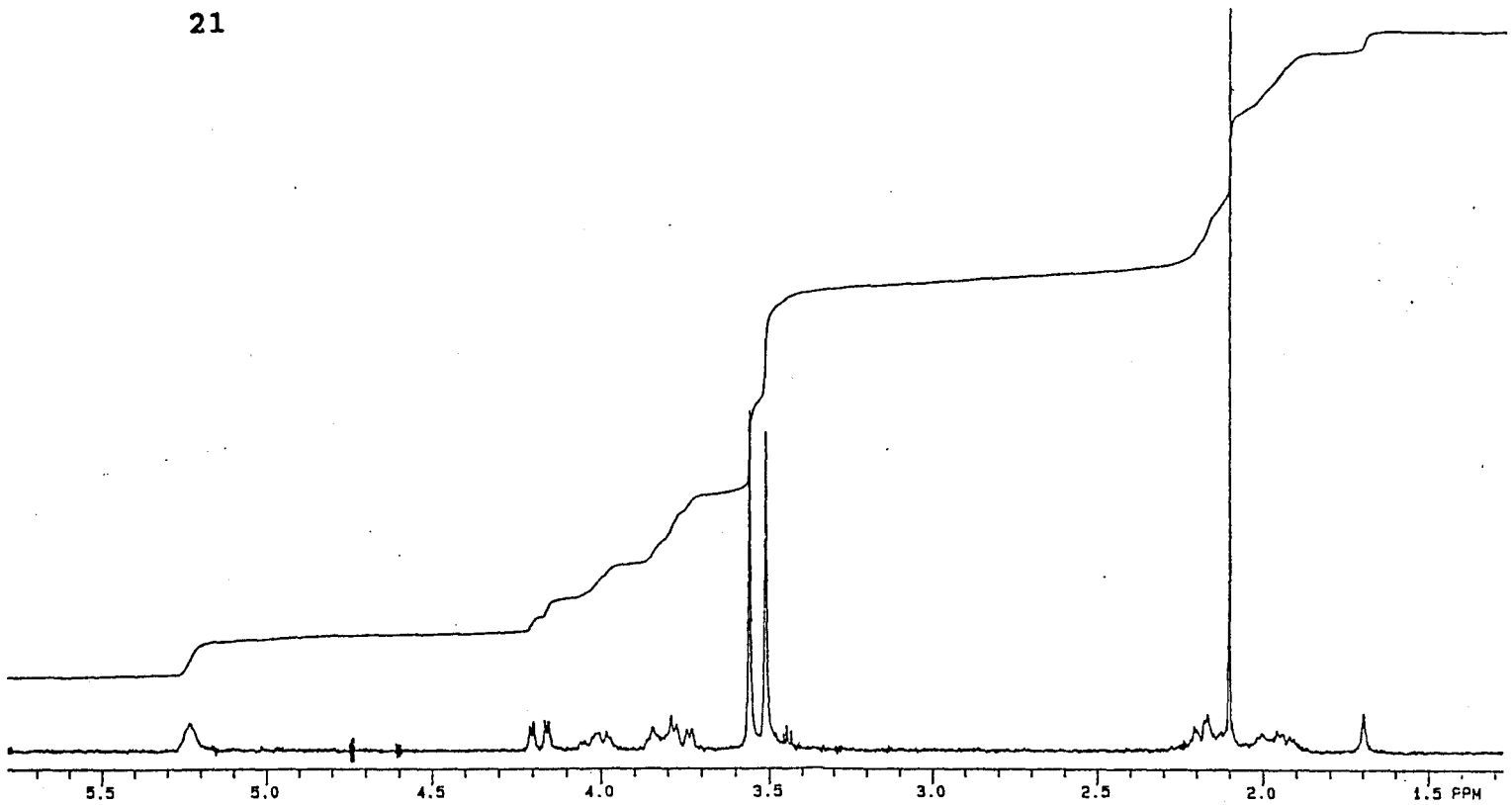
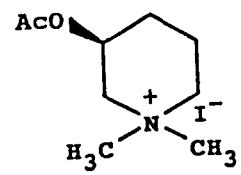


^{1}H NMR Spectrum of (S)-(-)-N,N-Dimethyl-3-acetoxypiperidinium Iodide (**21**)



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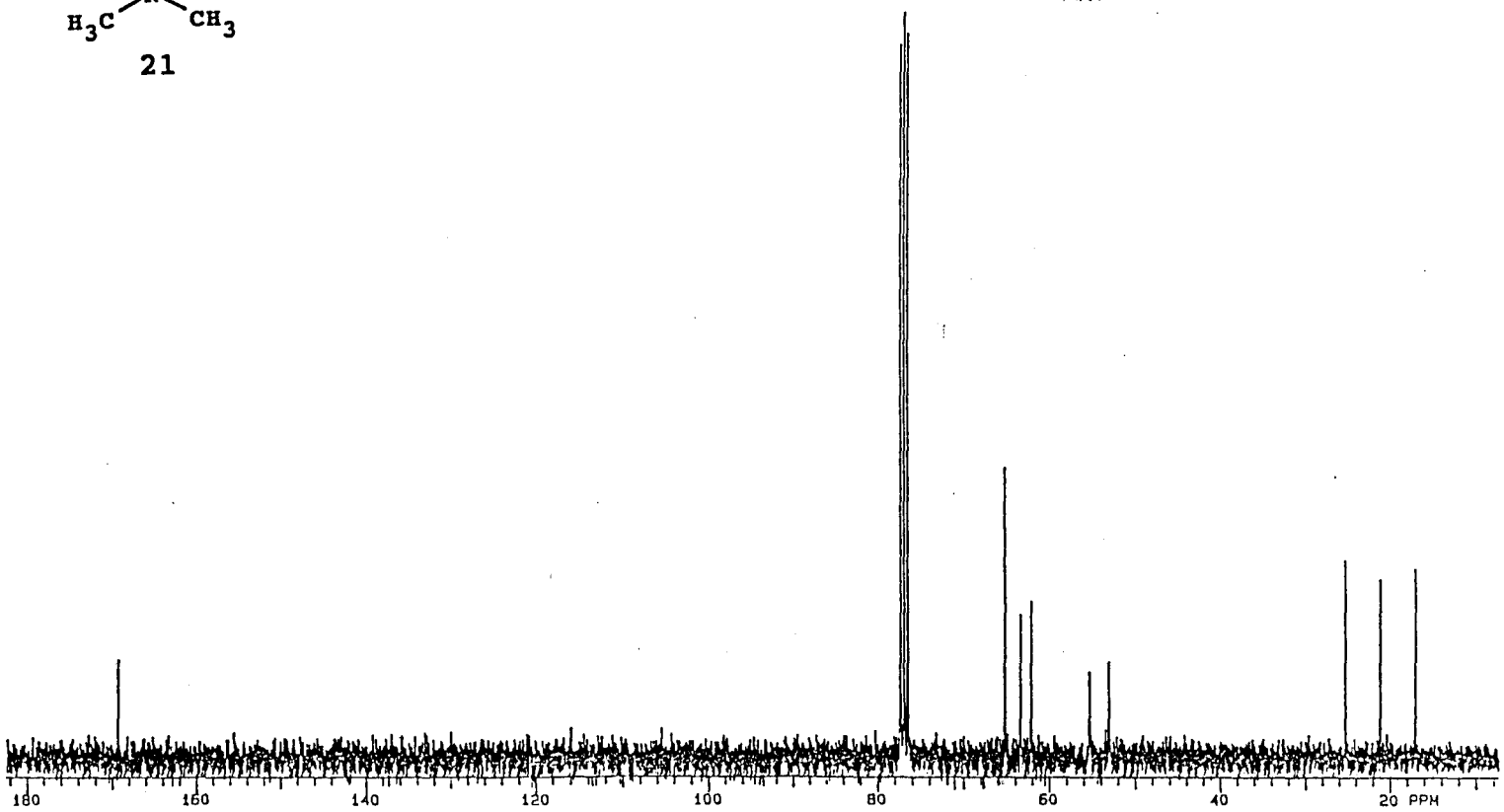
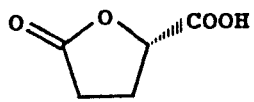


^{13}C NMR Spectrum of (S)-(-)-N,N-Dimethyl-3-acetoxypiperidinium Iodide (**21**)



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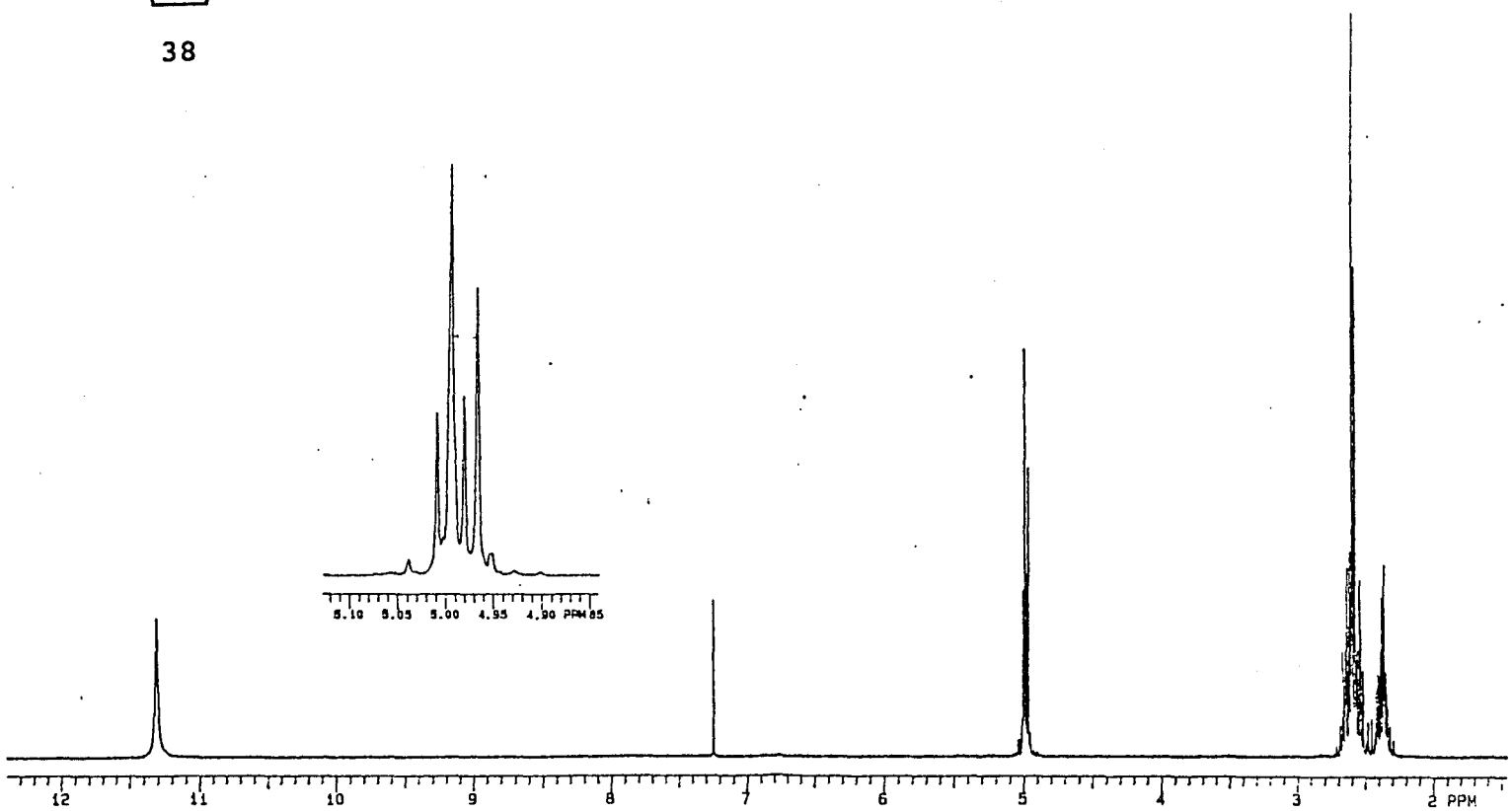
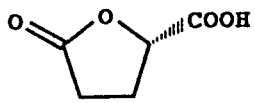
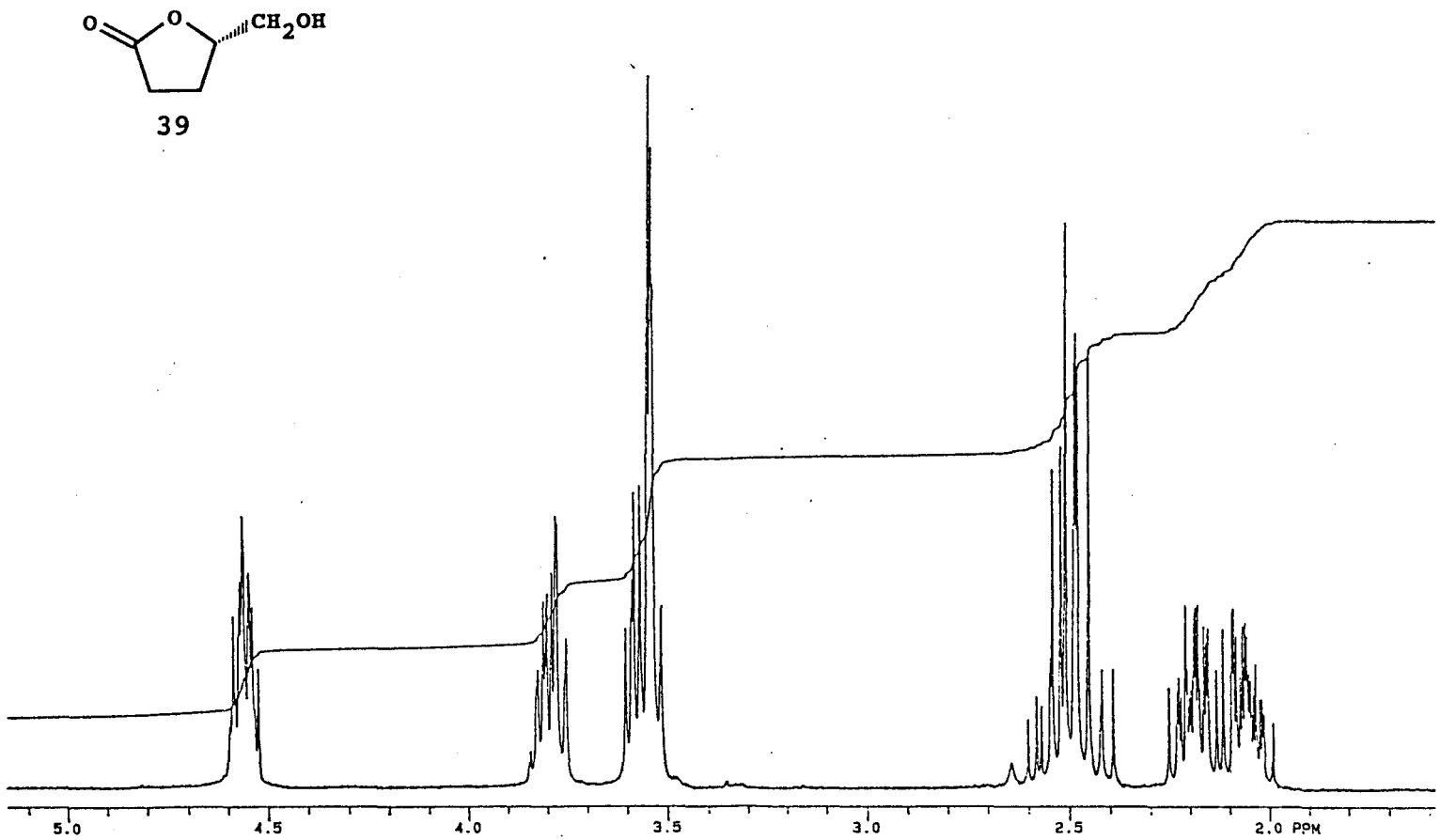


^{1}H NMR Spectrum of (S)-(+)-5-Oxo-2-tetrahydrofurancarboxylic acid (**38**)

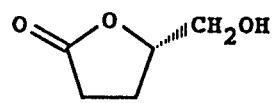


38

^{13}C NMR Spectrum of (S)-(+)-5-Oxo-2-tetrahydrofurancarboxylic acid (**38**)



^{1}H NMR Spectrum of (S)-(+)-γ-Hydroxymethyl-γ-butyrolactone (**39**)



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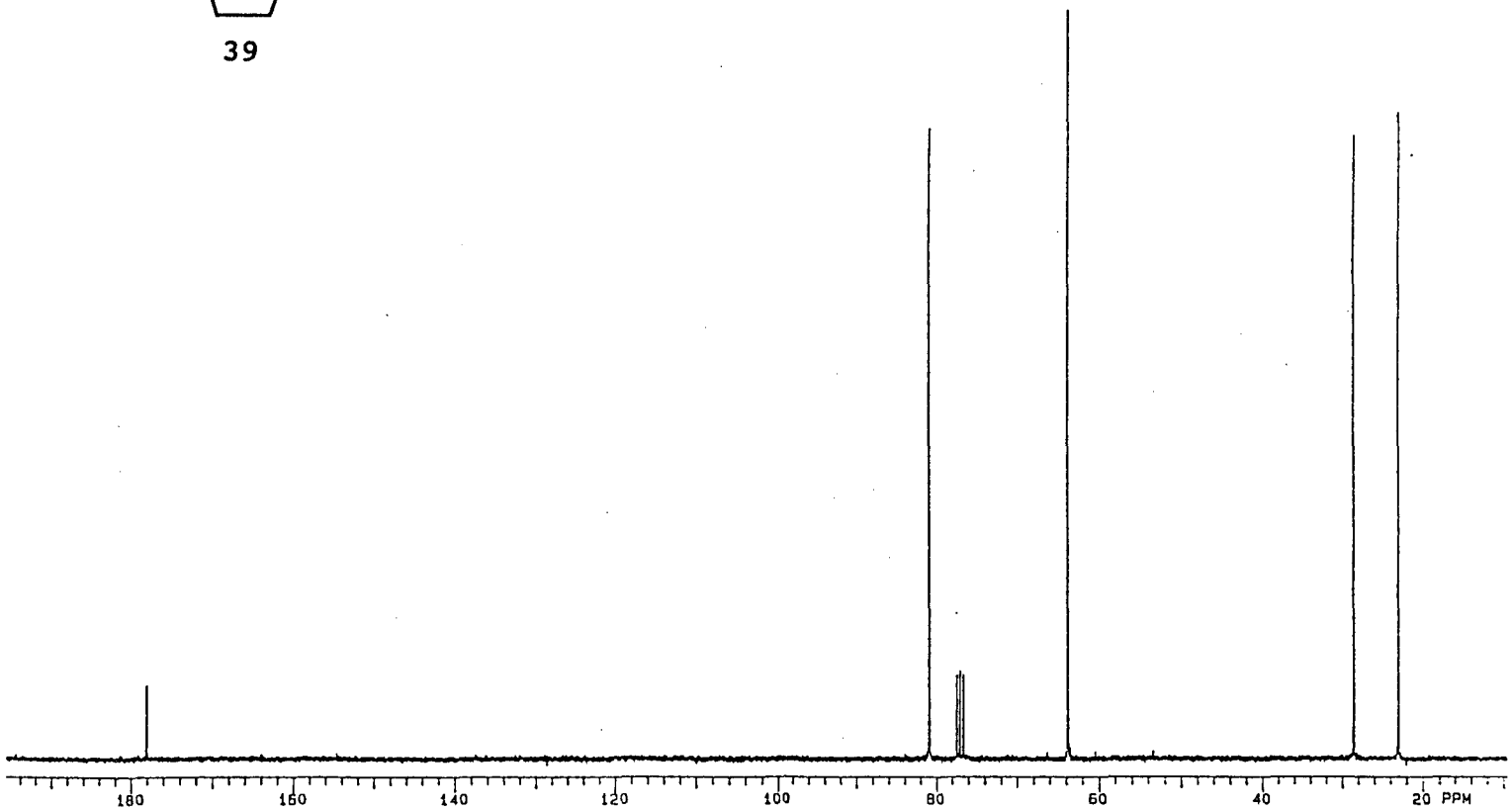
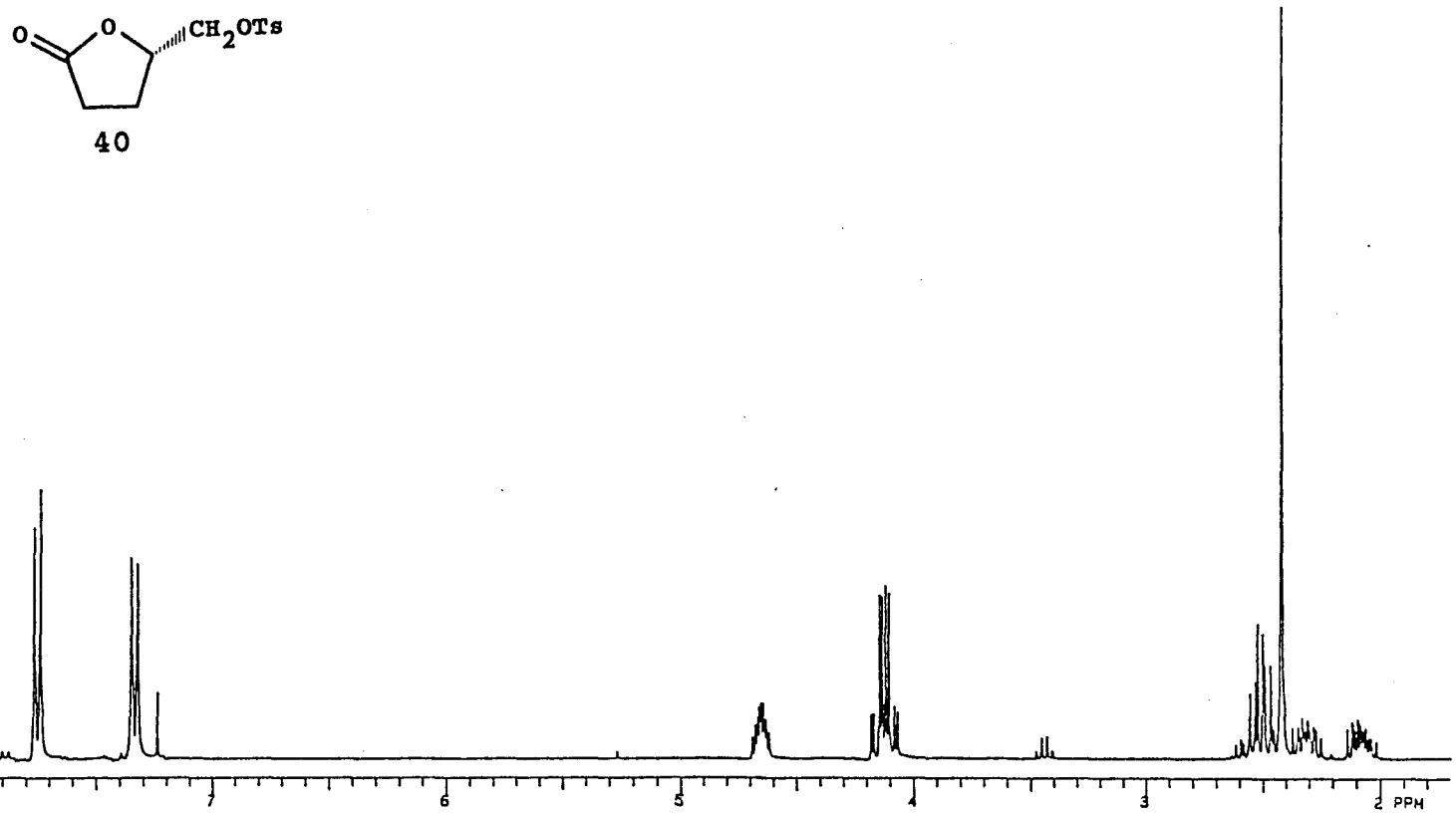
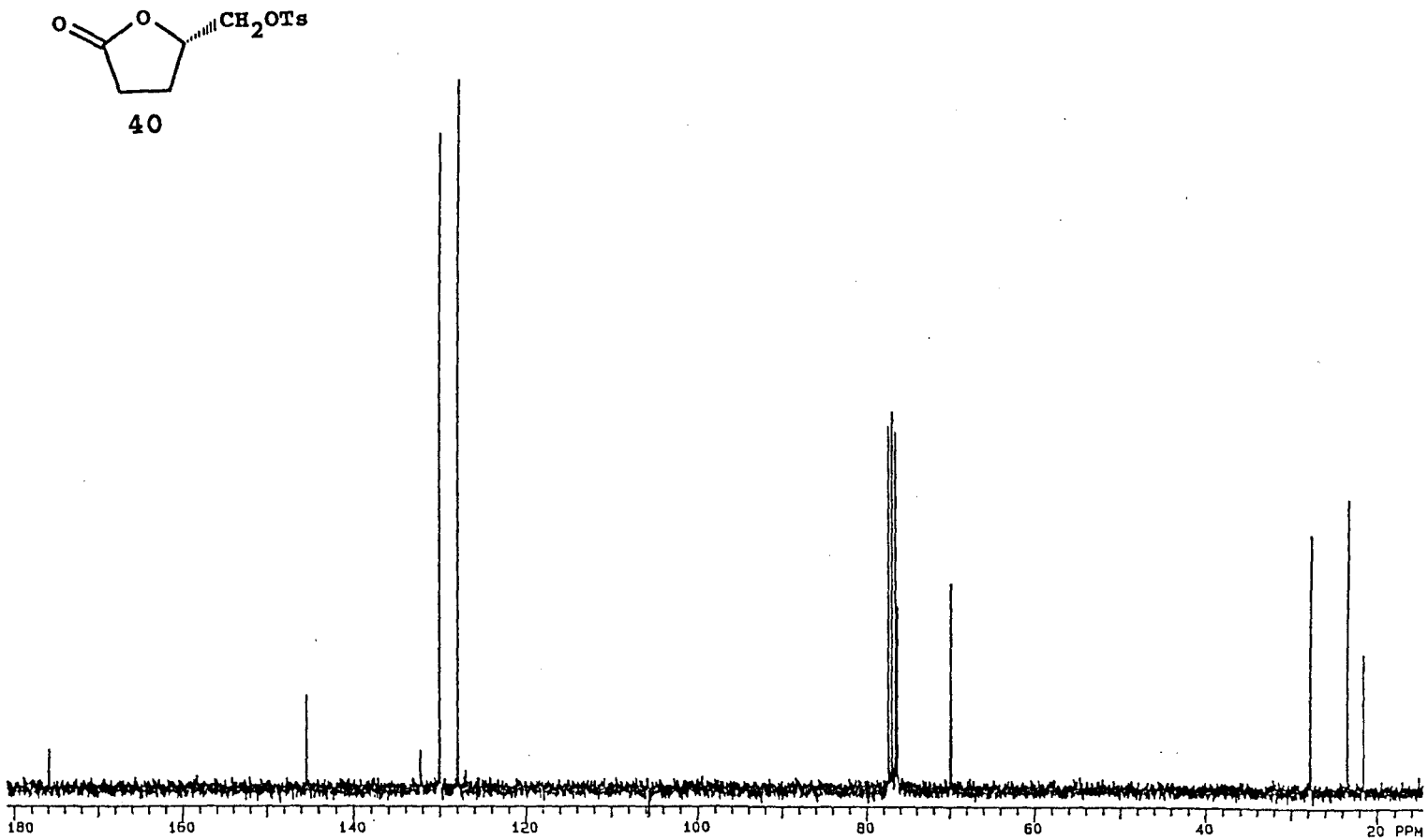


^{13}C NMR Spectrum of (S)-(+)-γ-Hydroxymethyl-γ-butyrolactone (**39**)

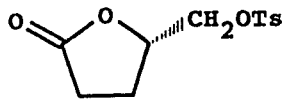


40

^{1}H NMR Spectrum of (S)-(+)-γTosyloxymethyl-γ-butyrolactone (**40**)



^{13}C NMR Spectrum of (S)-(+)-γTosyloxymethyl-γ-butyrolactone (**40**)



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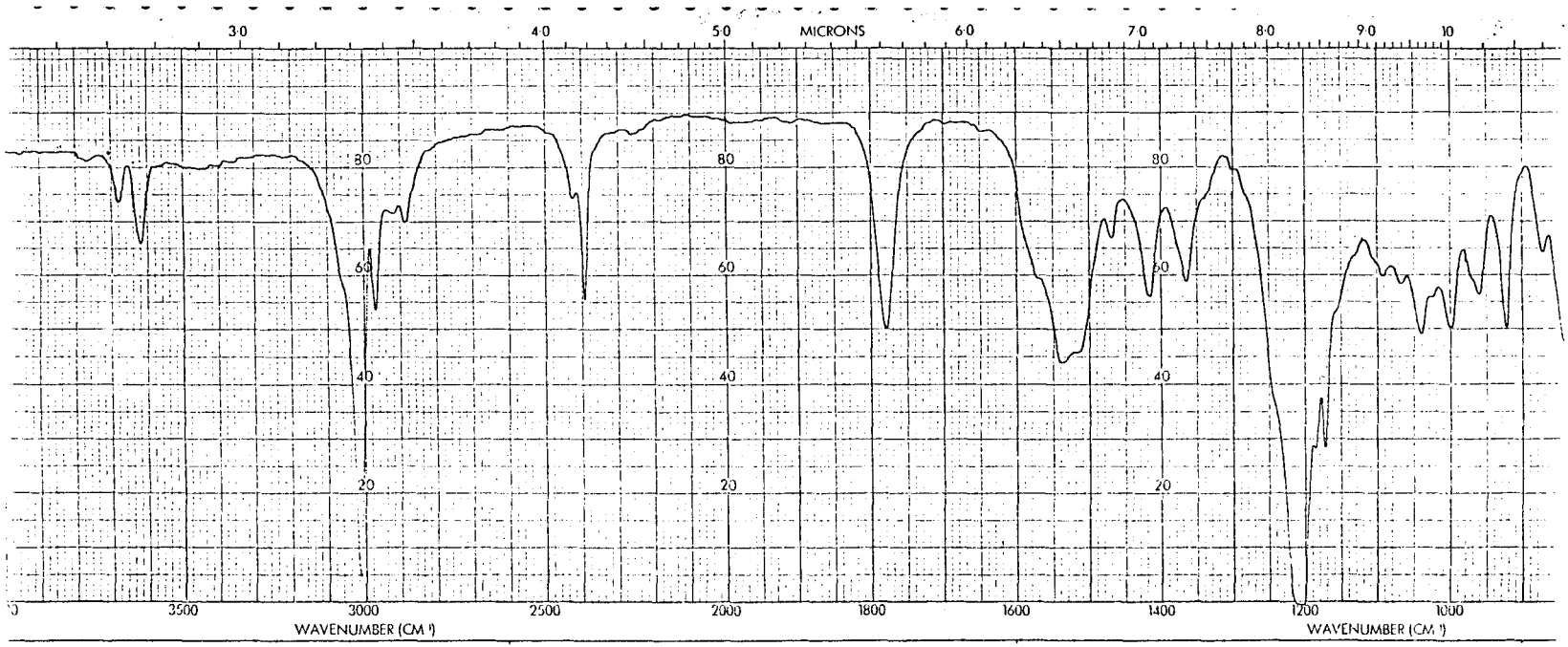
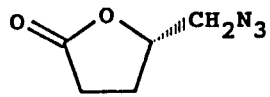


IR Spectrum of (S)-(+)-γTosyloxymethyl-γ-butyrolactone (**40**)



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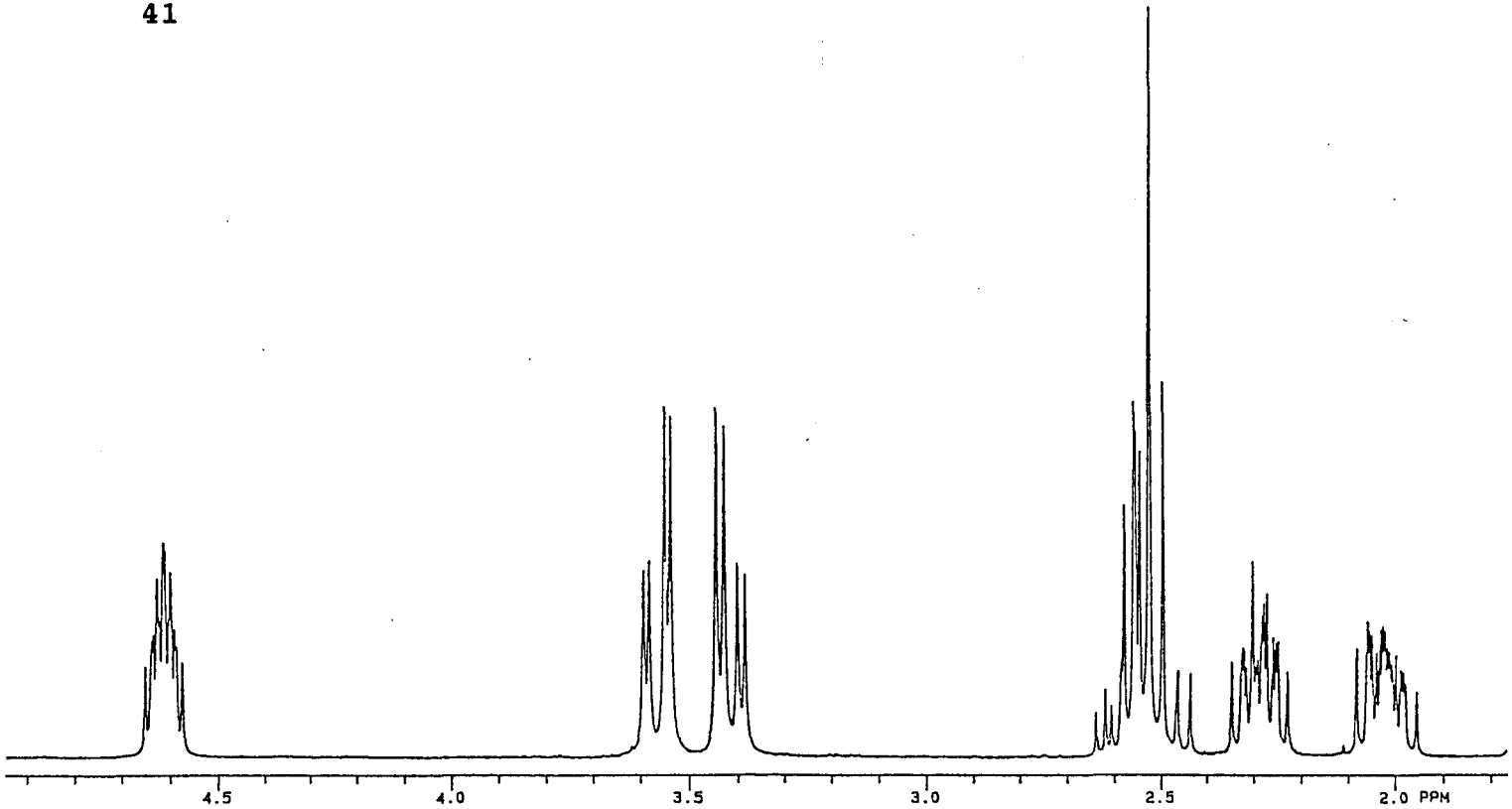
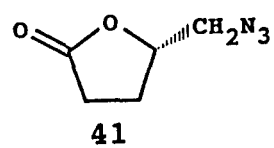


^{1}H NMR Spectrum of (S)-(+)-5-Azido-4-pentanolide (**41**)



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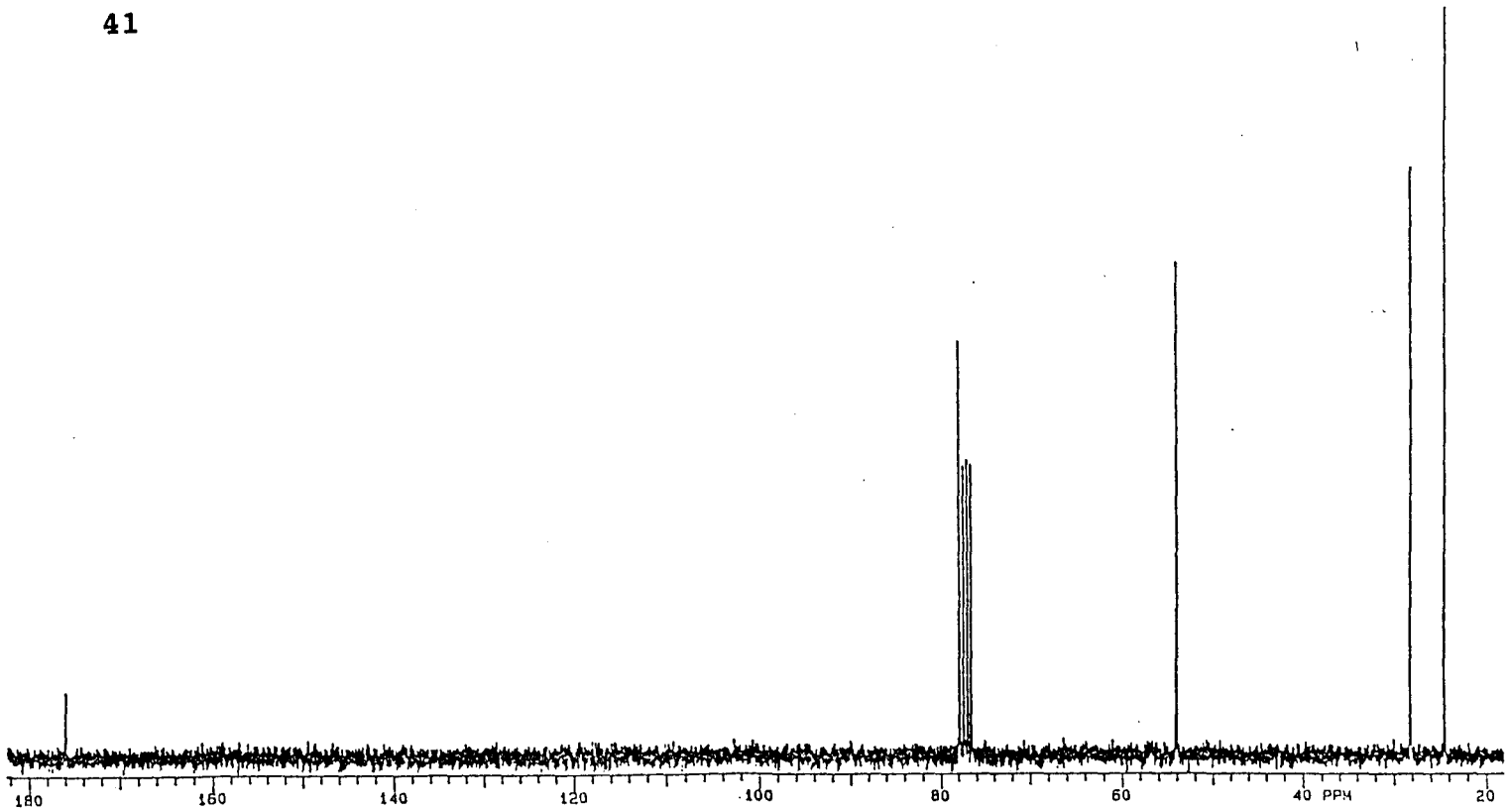
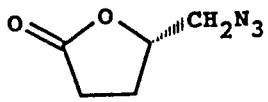


^{13}C NMR Spectrum of (S)-(+)-5-Azido-4-pentanolide (**41**)



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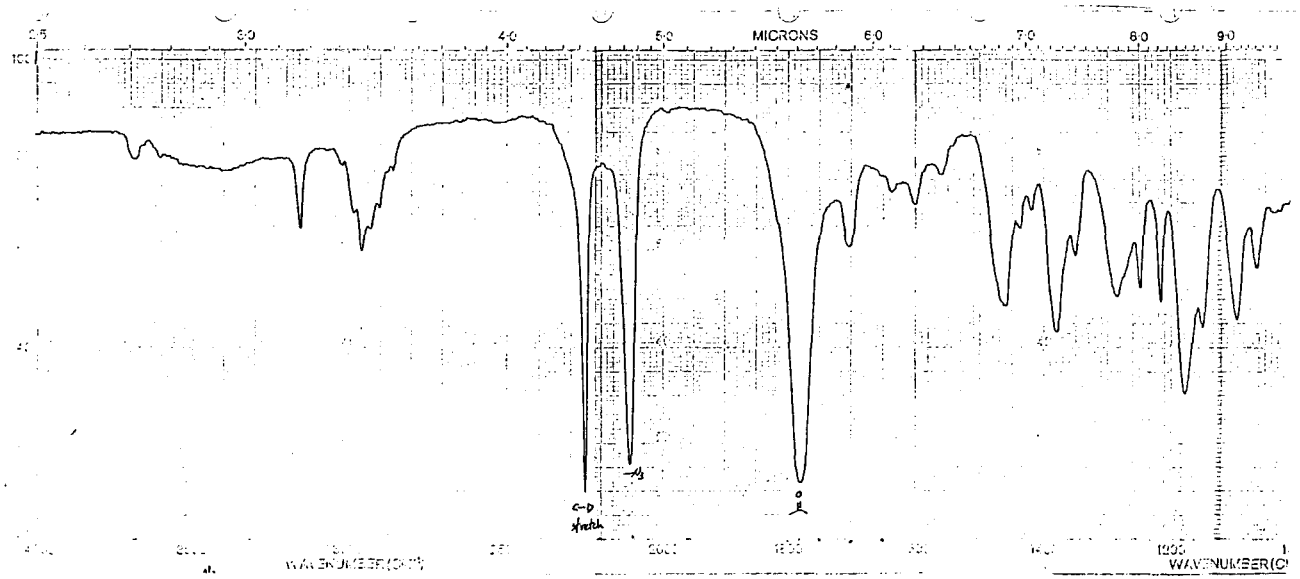
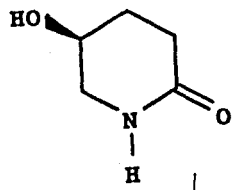
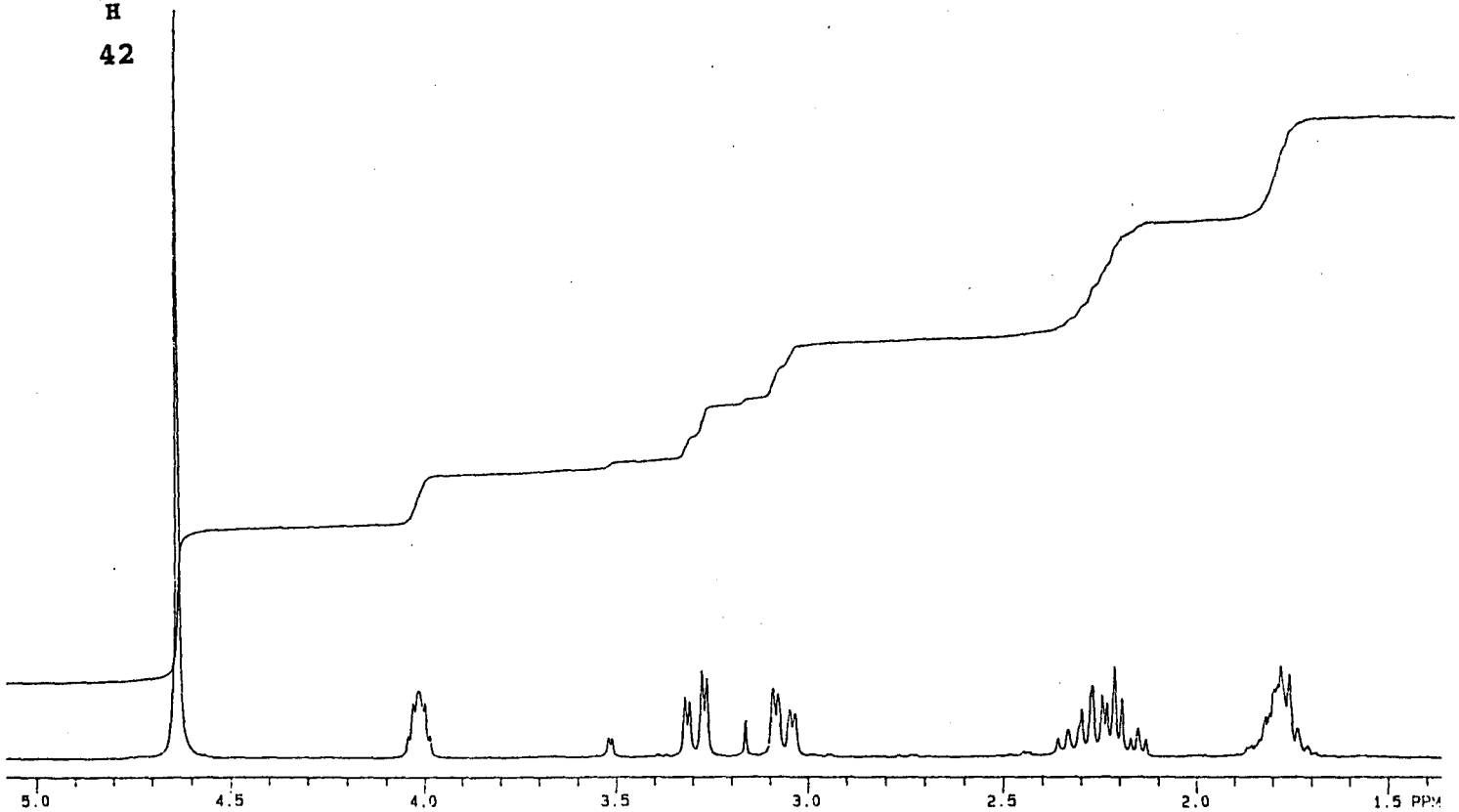


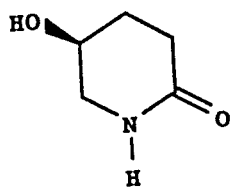
IR Spectrum of (S)-(+)-5-Azido-4-pentanolide (**41**)



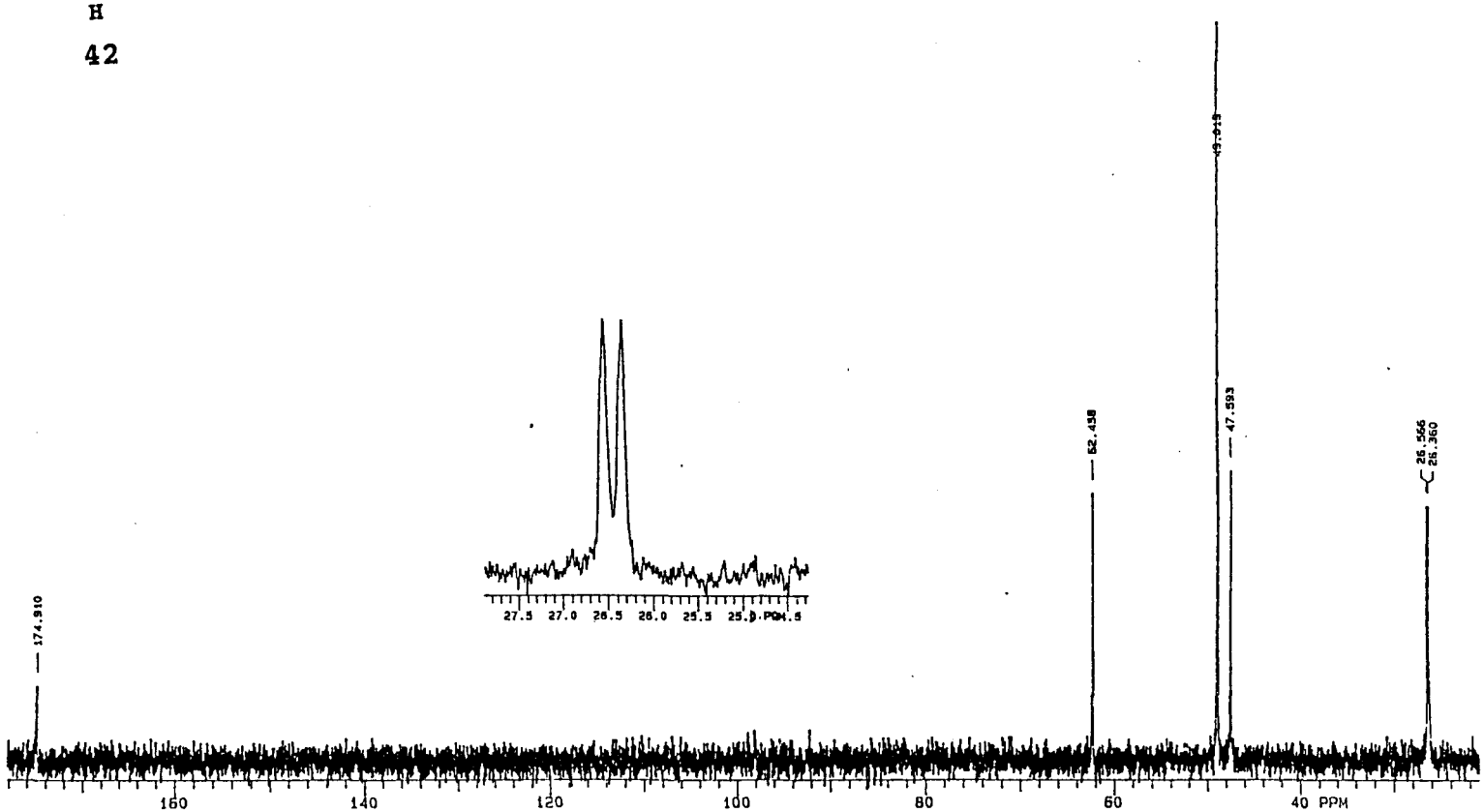
42



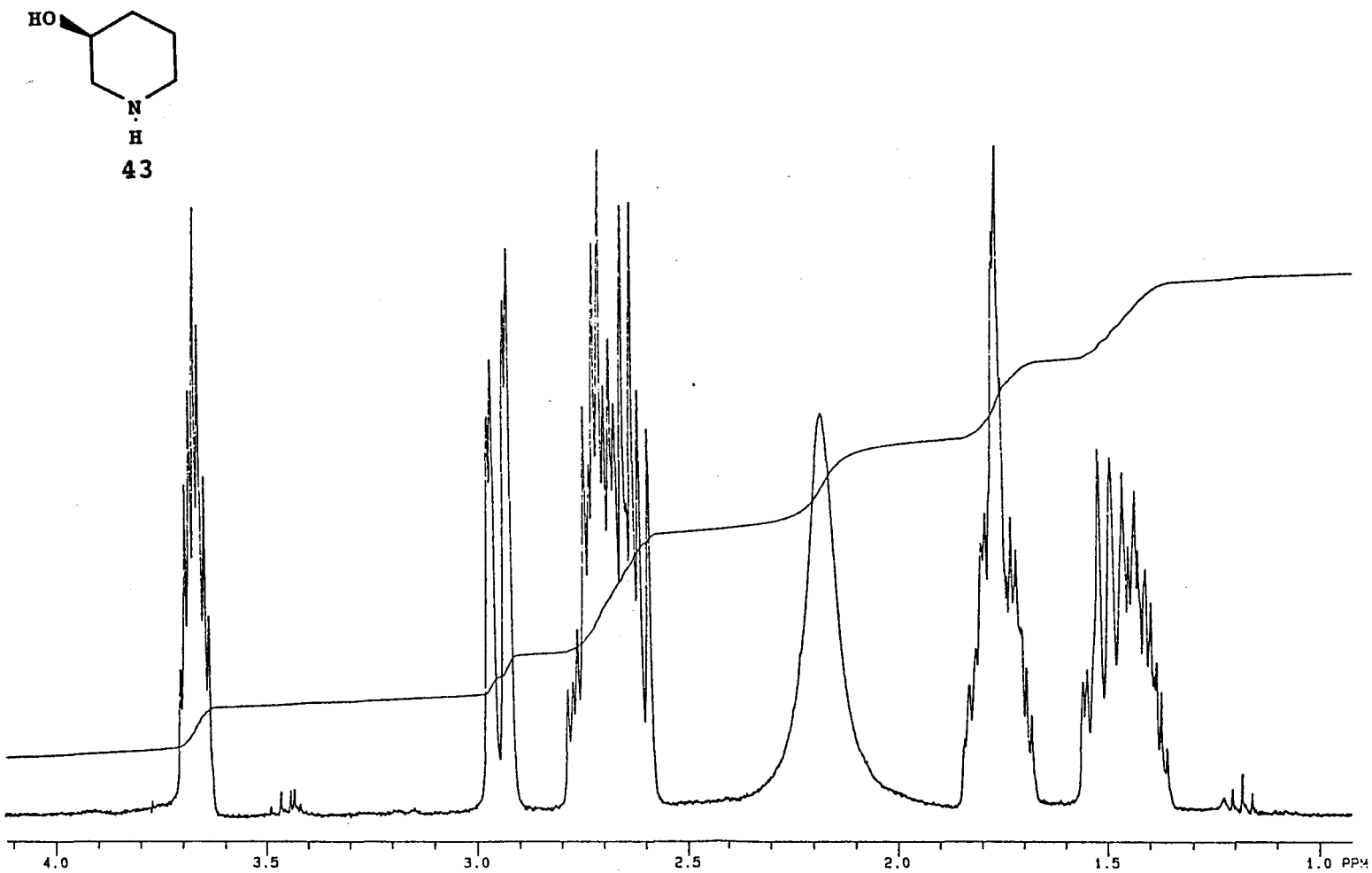
^{1}H NMR Spectrum of (S)-(-)-5-Hydroxy-2-piperidinone (**42**)



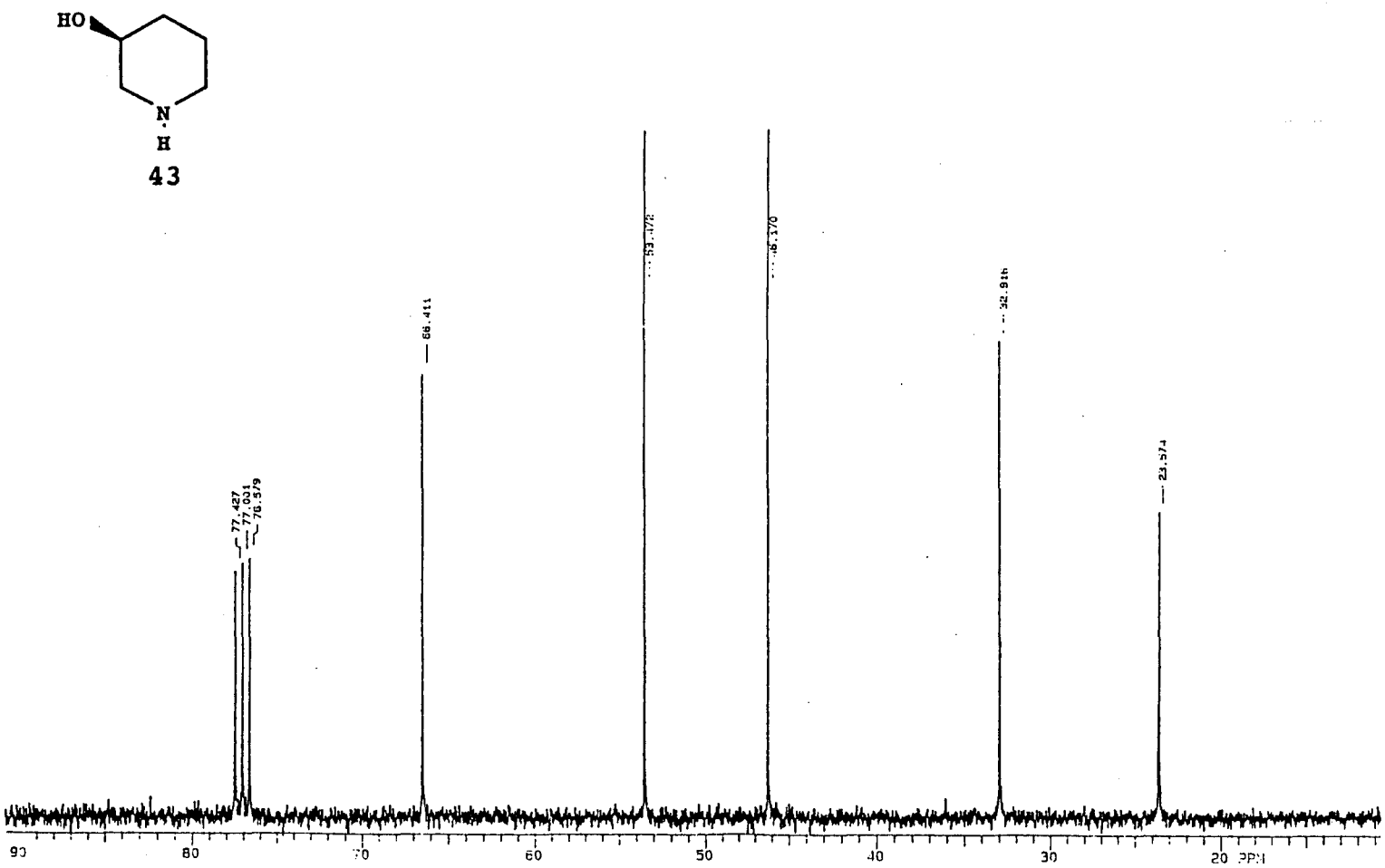
42



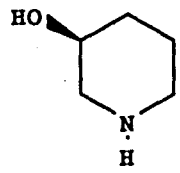
^{13}C NMR Spectrum of (S)-(-)-5-Hydroxy-2-piperidinone (**42**)



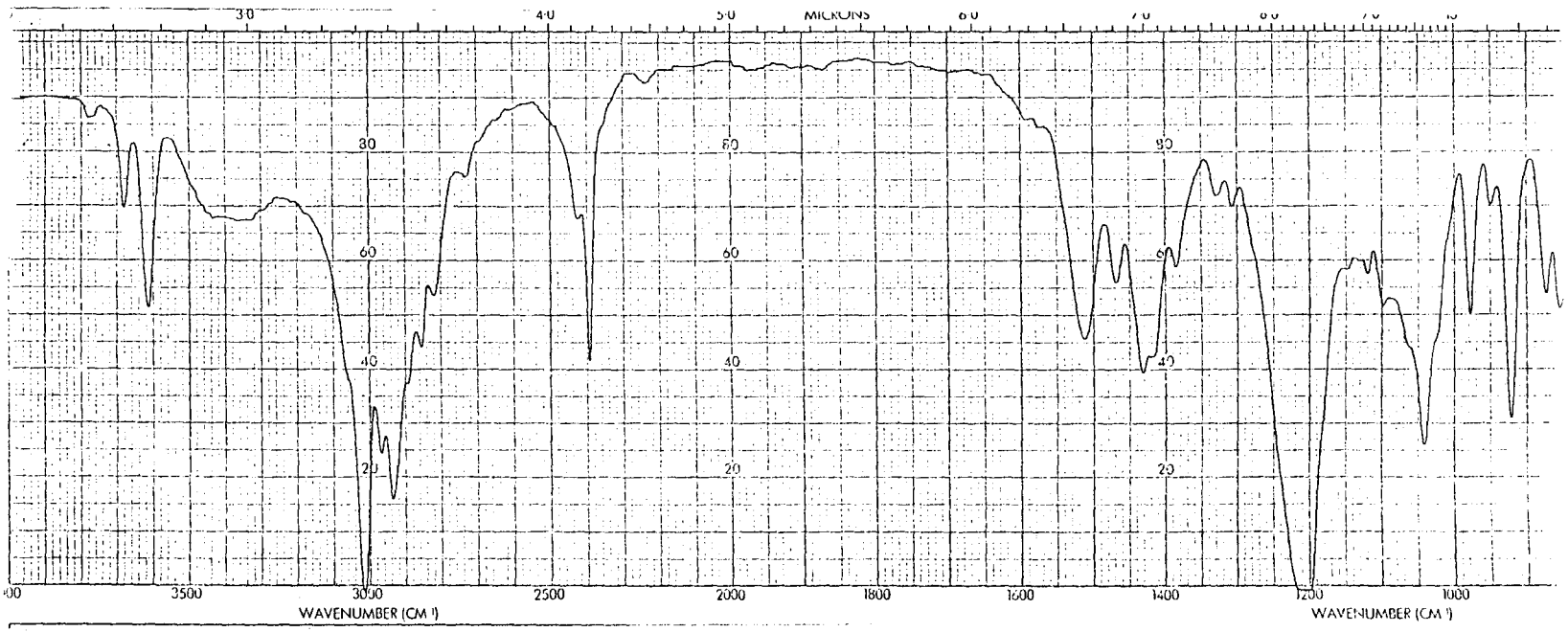
^{1}H NMR Spectrum of (S)-(-)-3-Hydroxypiperidine (**43**)



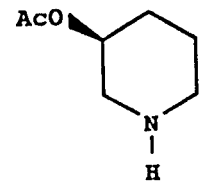
^{13}C NMR Spectrum of (S)-(-)-3-Hydroxypiperidine (**43**)



43



IR Spectrum of (S)-(-)-3-Hydroxypiperidine (**43**)



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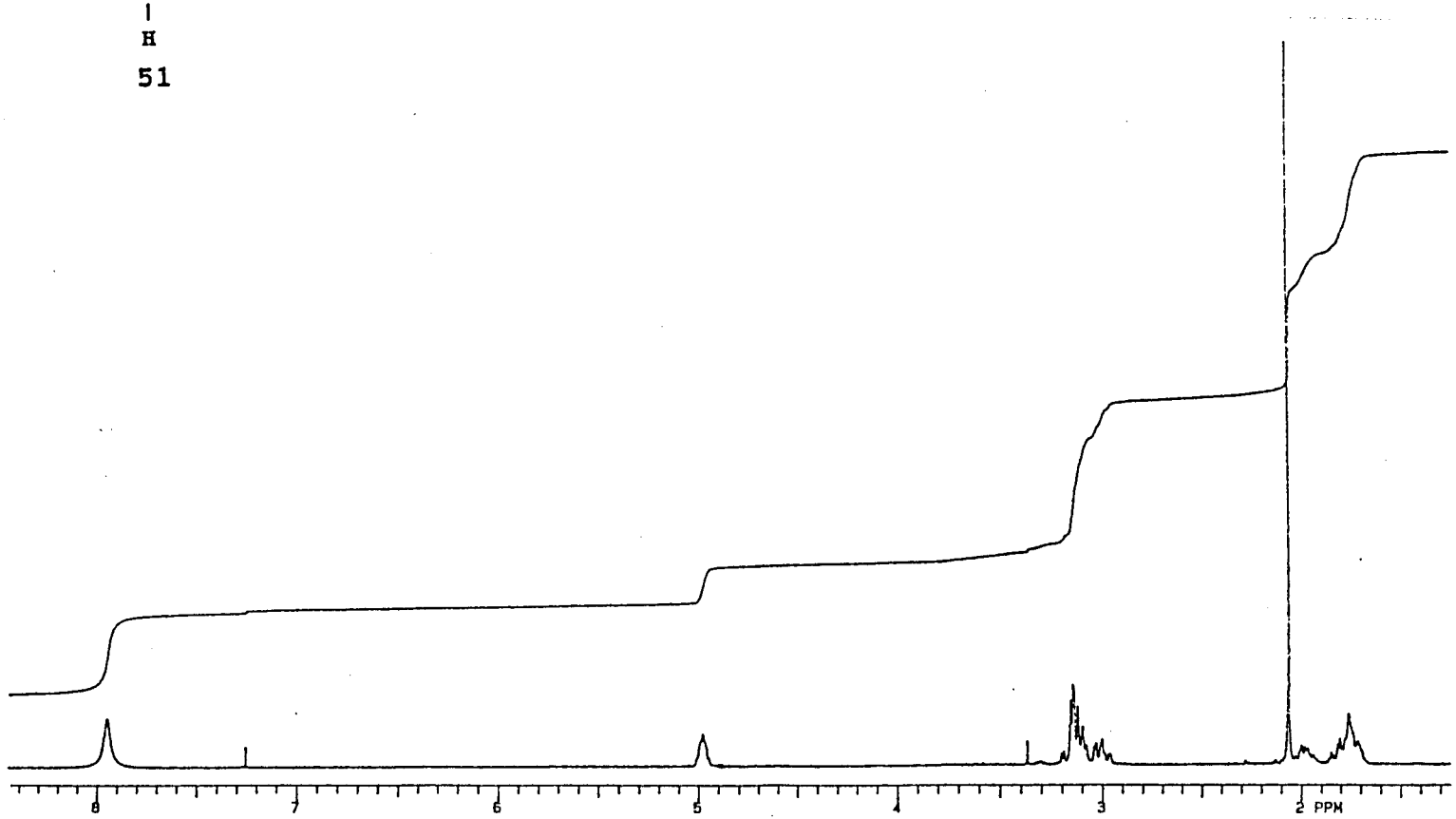
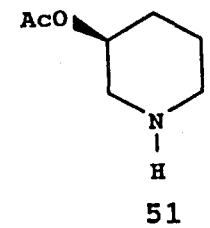
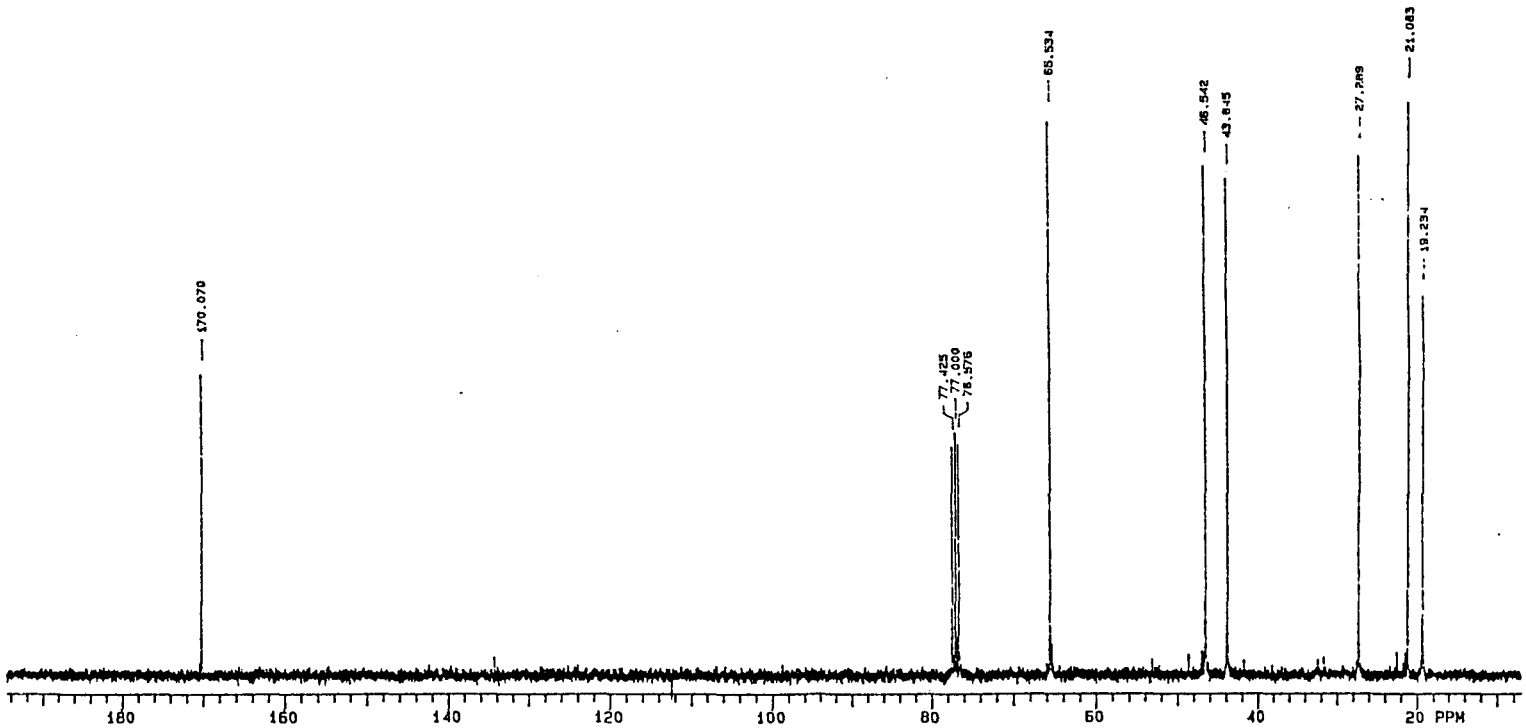


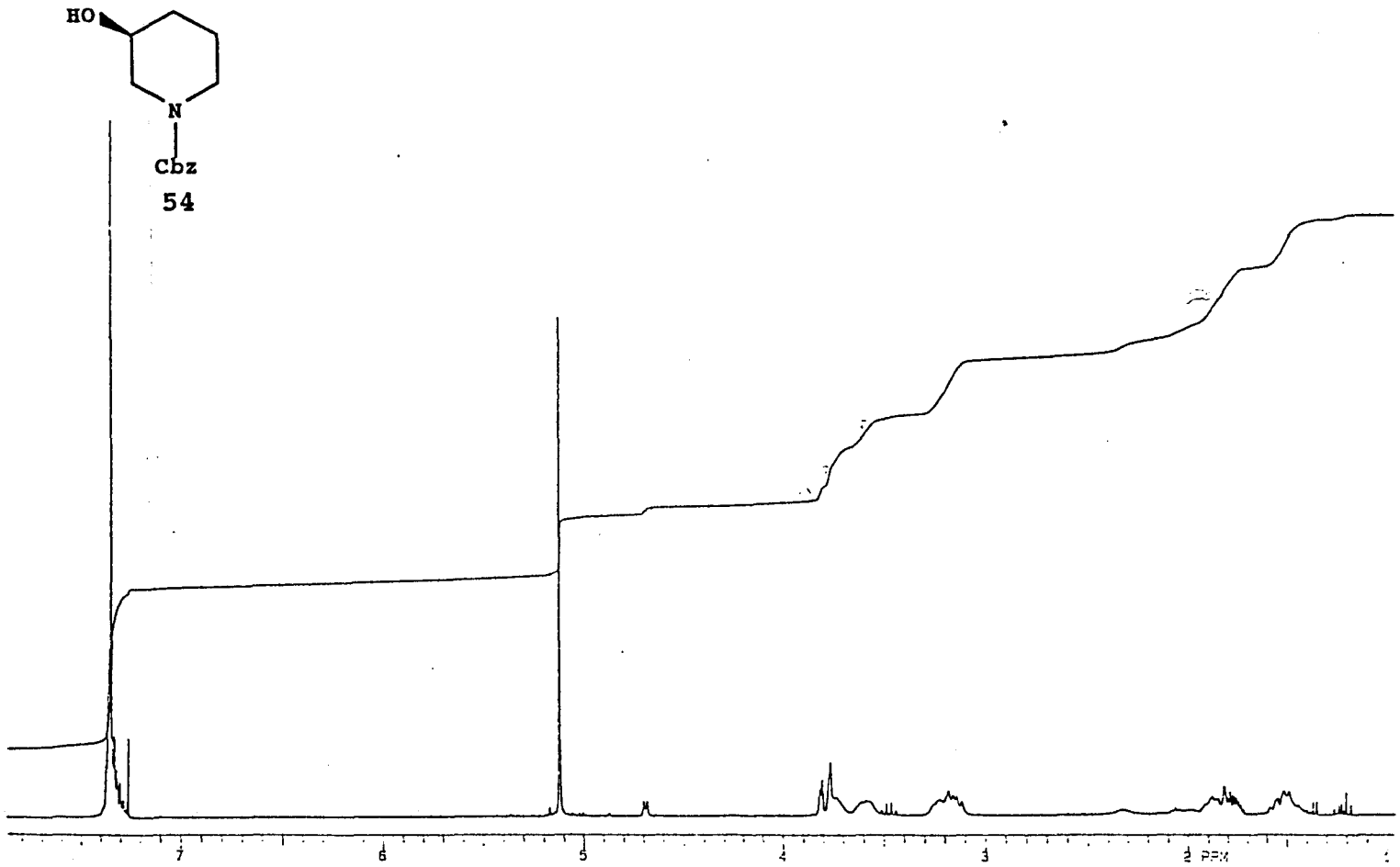
^{1}H NMR Spectrum of (S)-(-)-3-Acetoxypiperidine (**51**)



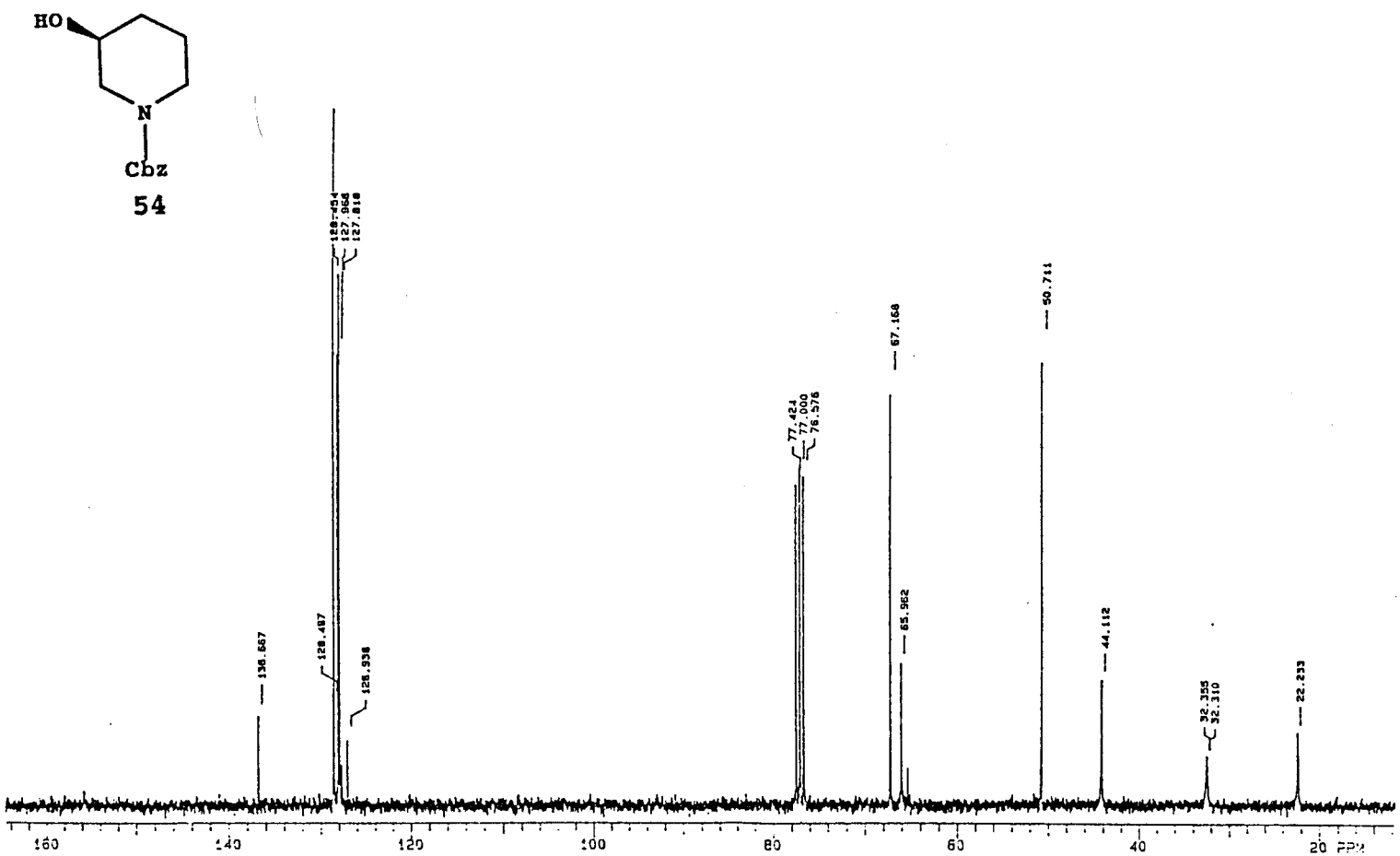
51



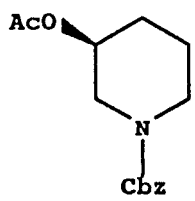
^{13}C NMR Spectrum of (S)-(-)-3-Acetoxypiperidine (**51**)



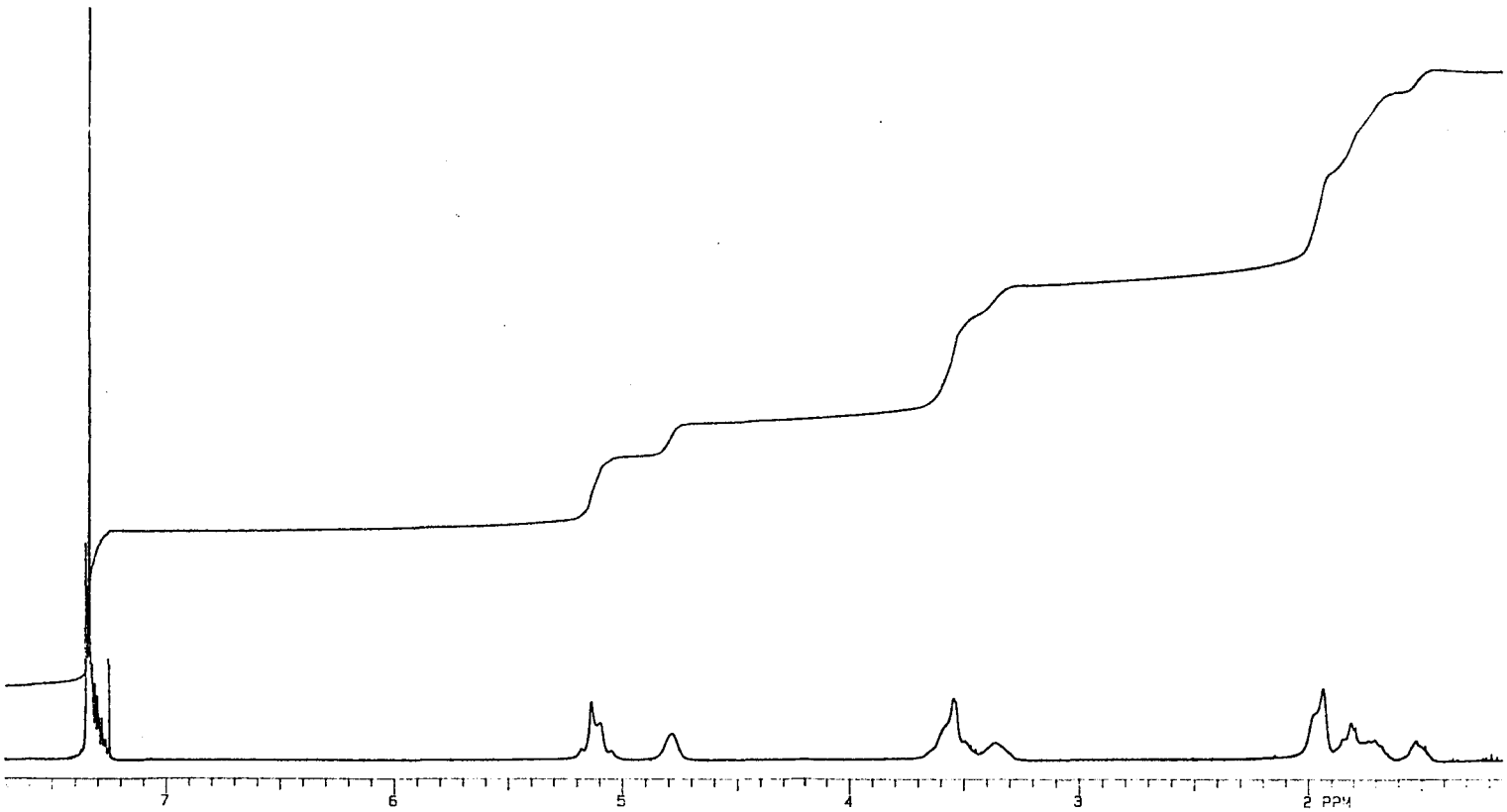
^{1}H NMR Spectrum of (S)-(+)-N-Cbz-3-Hydroxypiperidine (**54**)



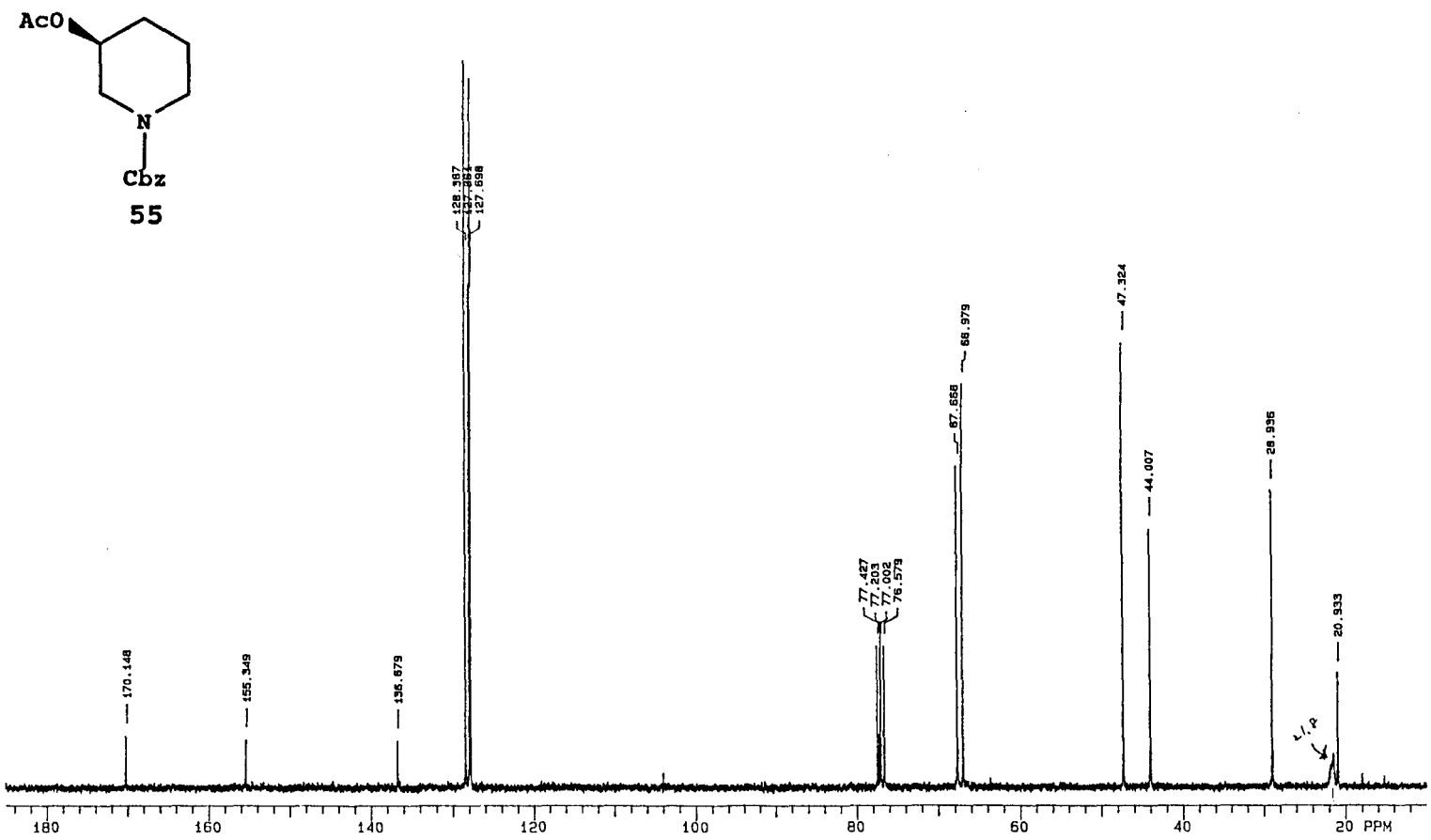
^{13}C NMR Spectrum of (S)-(+)-N-Cbz-3-Hydroxypiperidine (**54**)



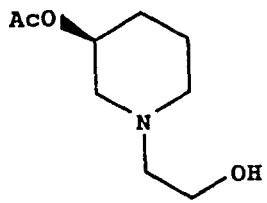
55



^{1}H NMR Spectrum of (S)-(-)-N-Cbz-3-Acetoxypiperidine (**55**)



^{13}C NMR Spectrum of (S)-(-)-N-Cbz-3-Acetoxypiperidine (**55**)



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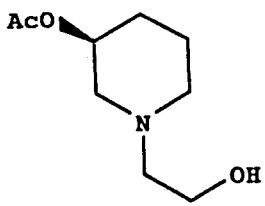
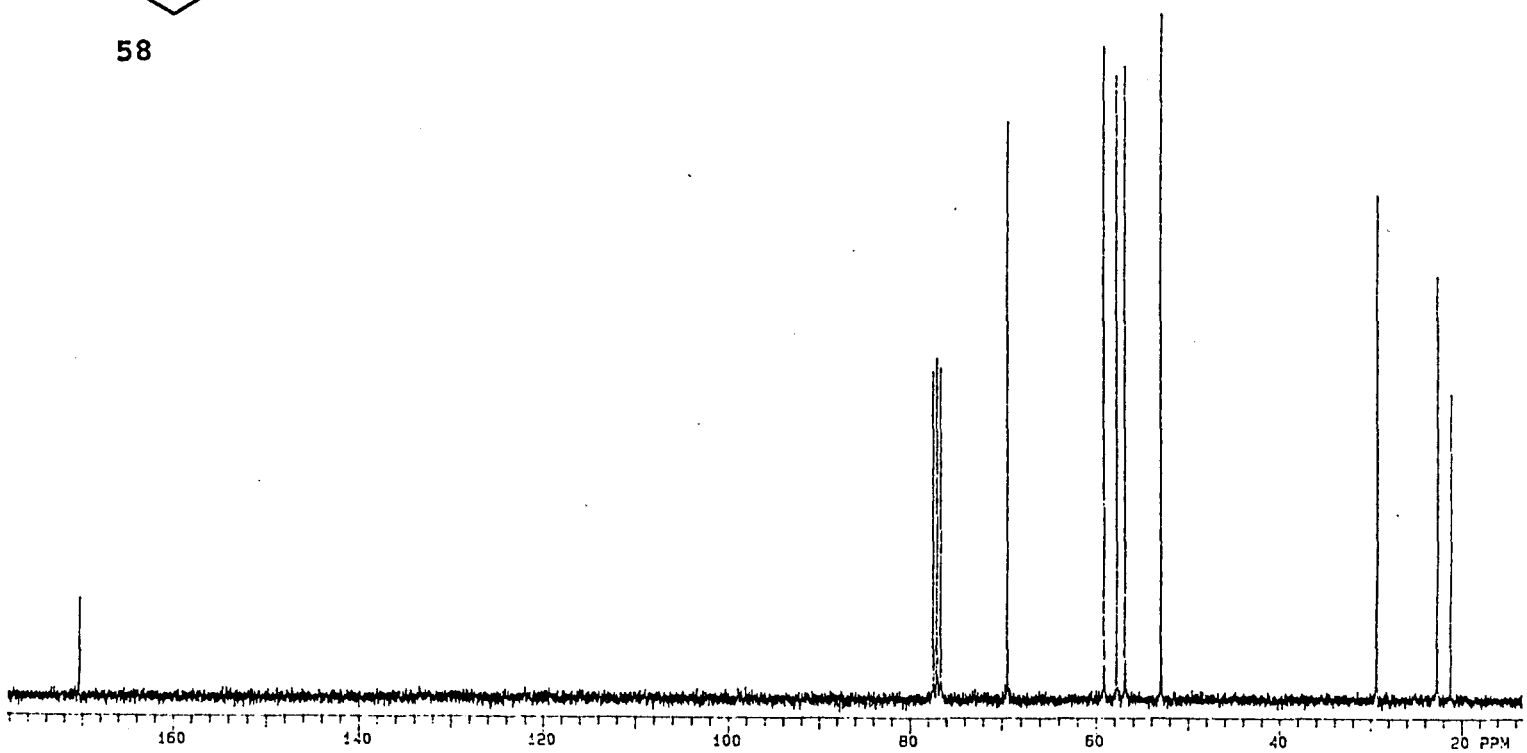
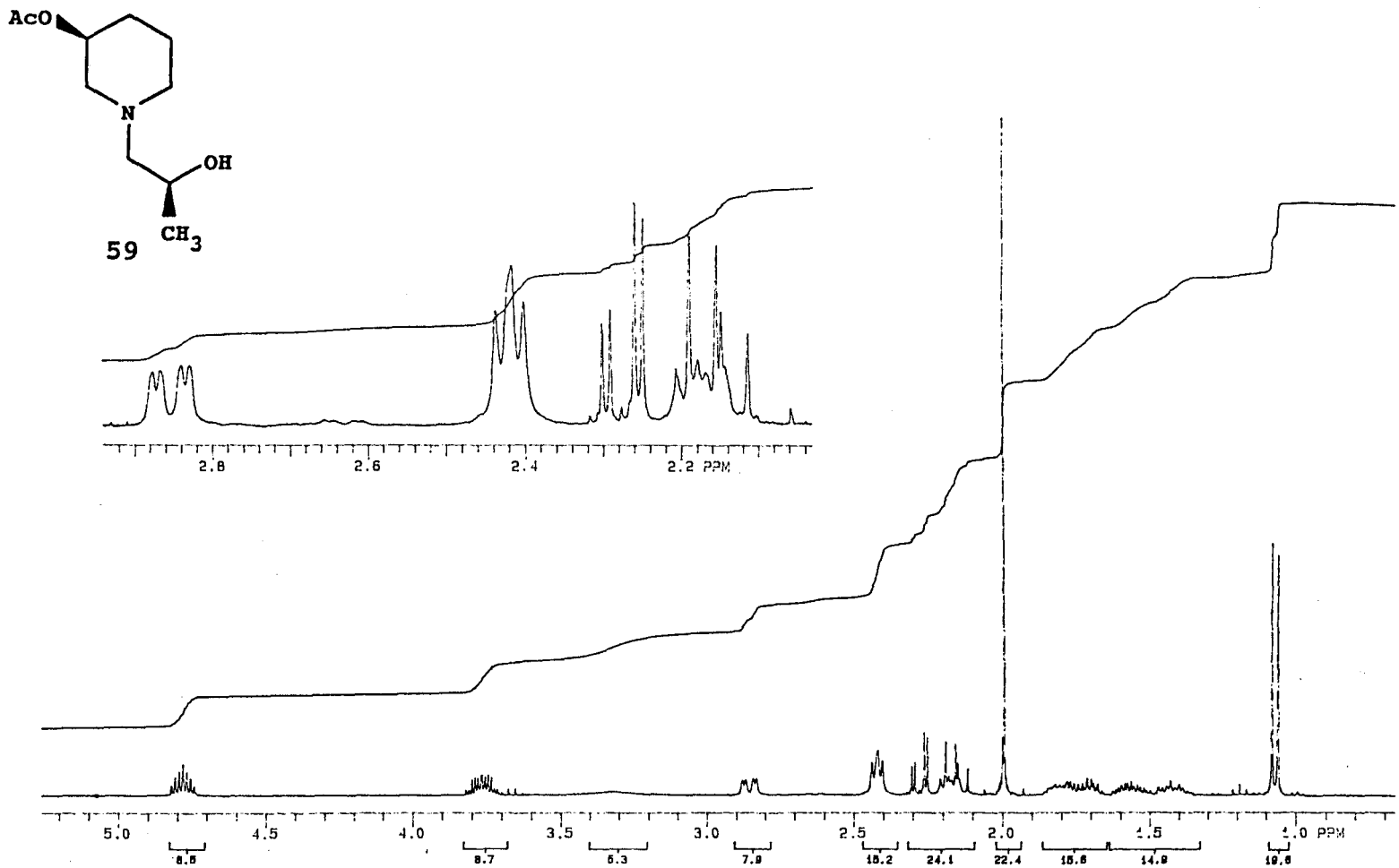
^{1}H NMR Spectrum of (S)-(-)-N-(2-Hydroxyethyl)-3-acetoxypiperidine (**58**)



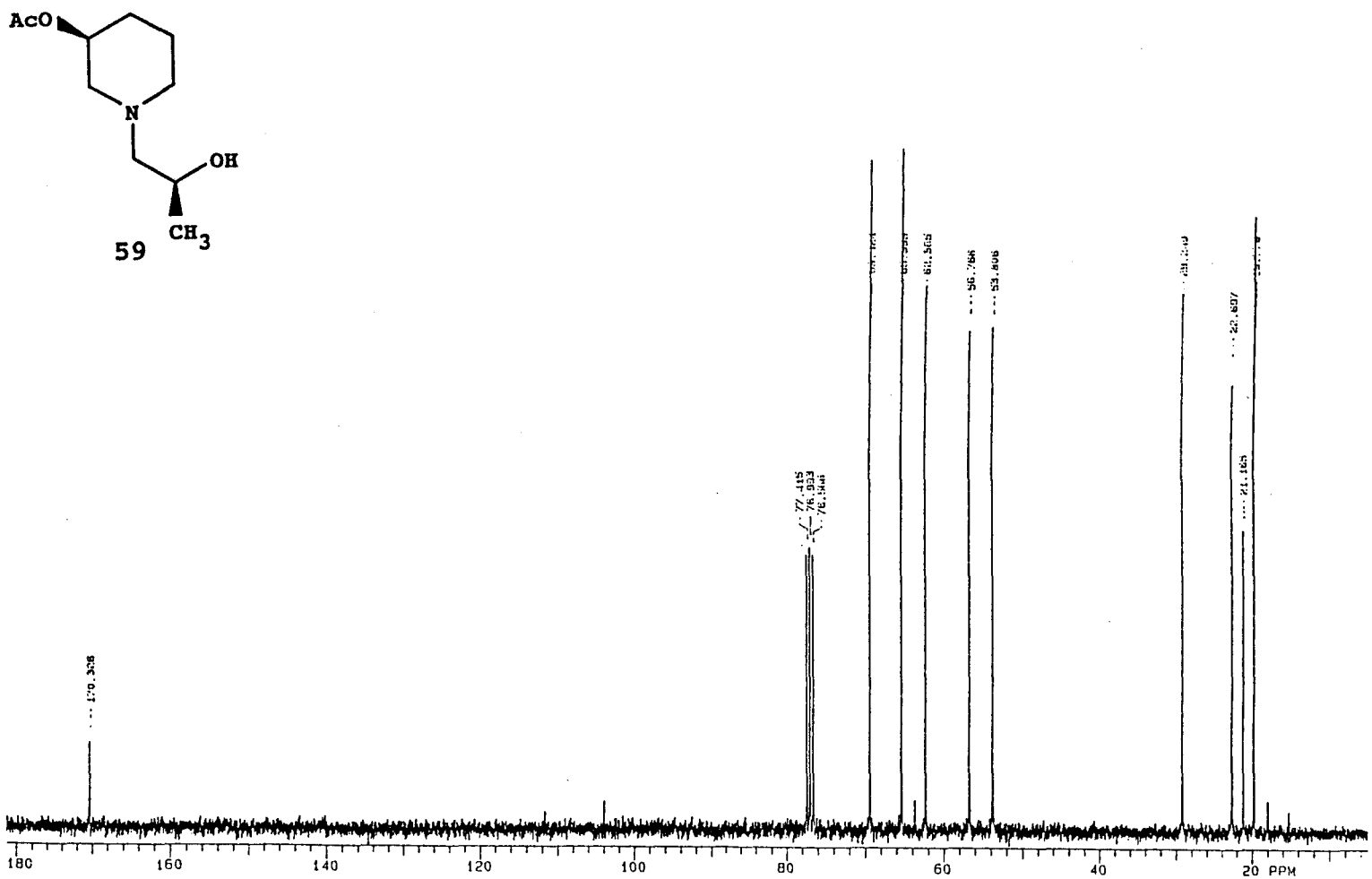
58



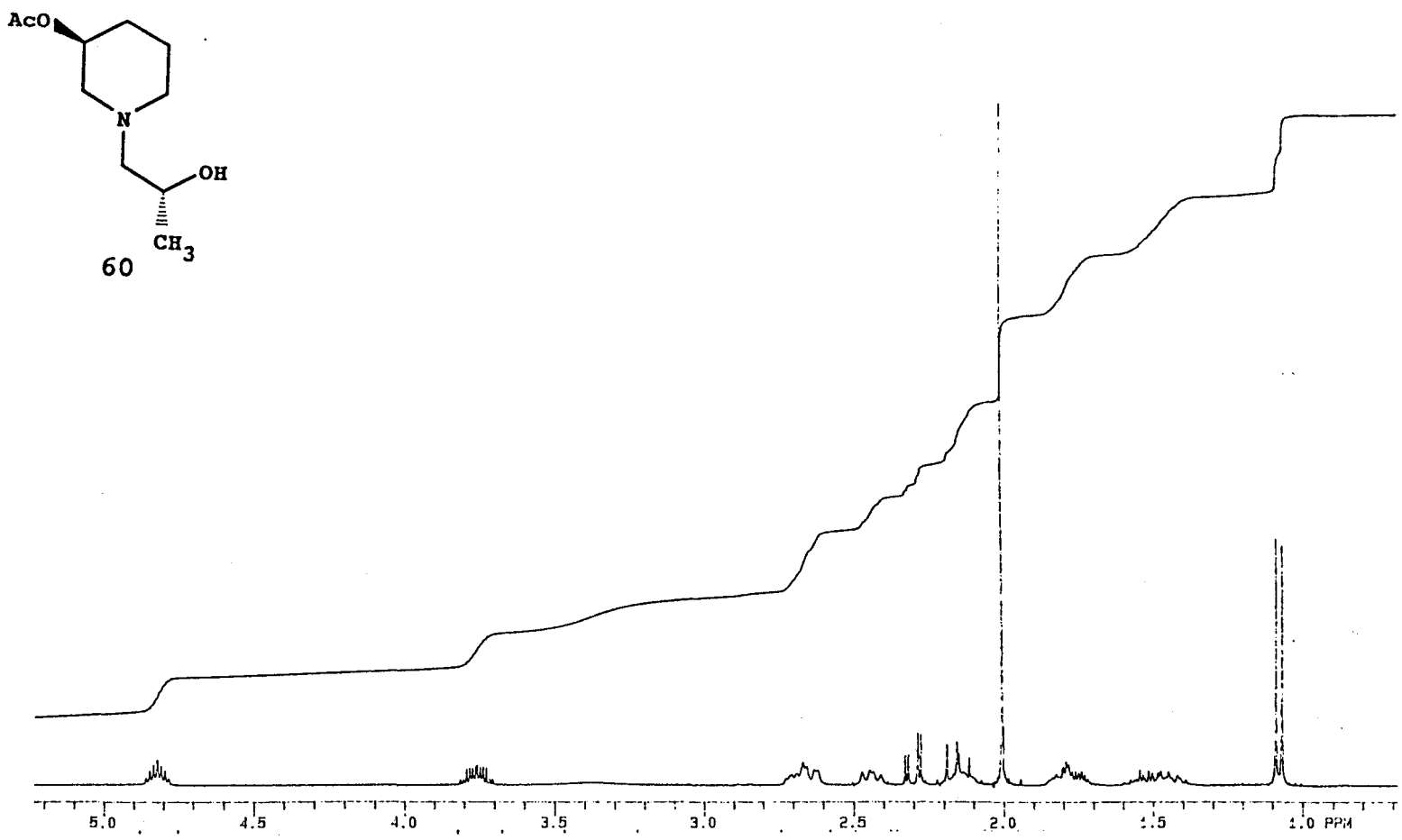
^{13}C NMR Spectrum of (S)-(-)-N-(2-Hydroxyethyl)-3-acetoxypiperidine (**58**)



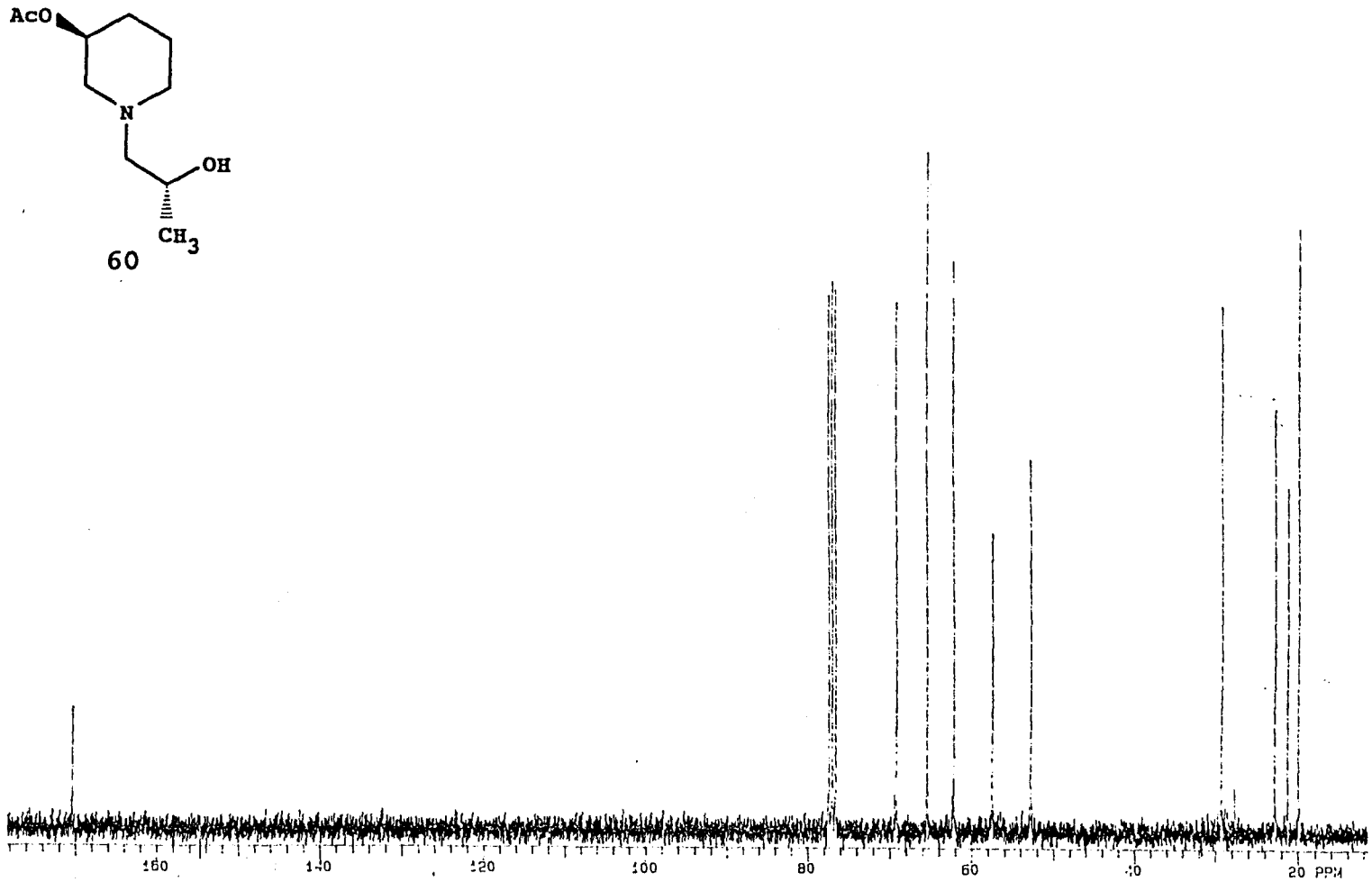
^{1}H NMR Spectrum of (S,S)-(+)-N-(2-Hydroxypropyl)-3-acetoxypiperidine (**59**)



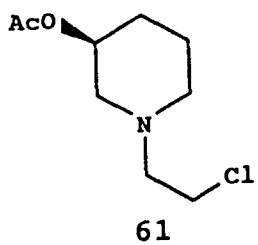
^{13}C NMR Spectrum of (S,S)-(+)-N-(2-Hydroxypropyl)-3-acetoxypiperidine (**59**)



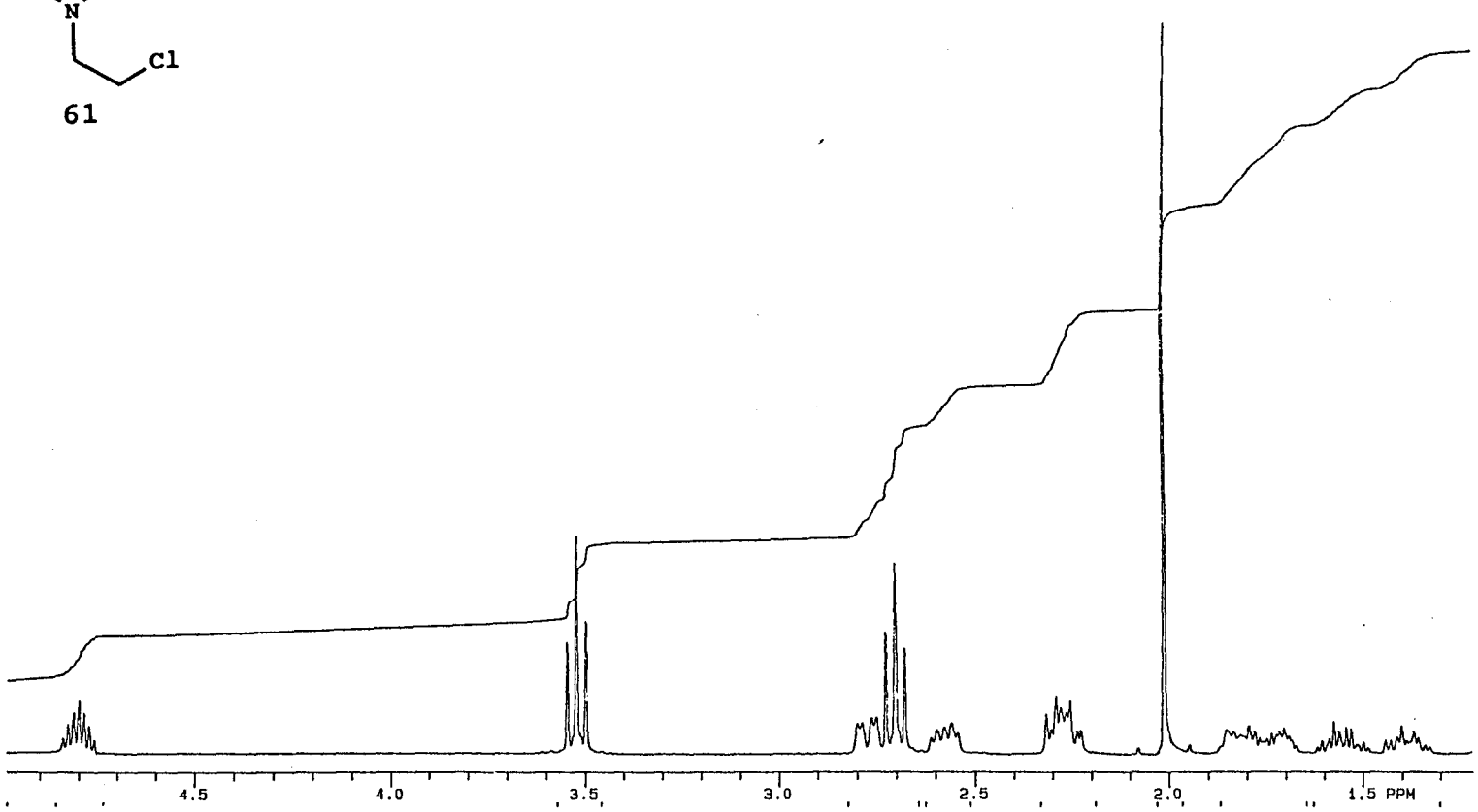
^{1}H NMR Spectrum of (R,S)-(-)-N-(2-Hydroxypropyl)-3-acetoxypiperidine (**60**)



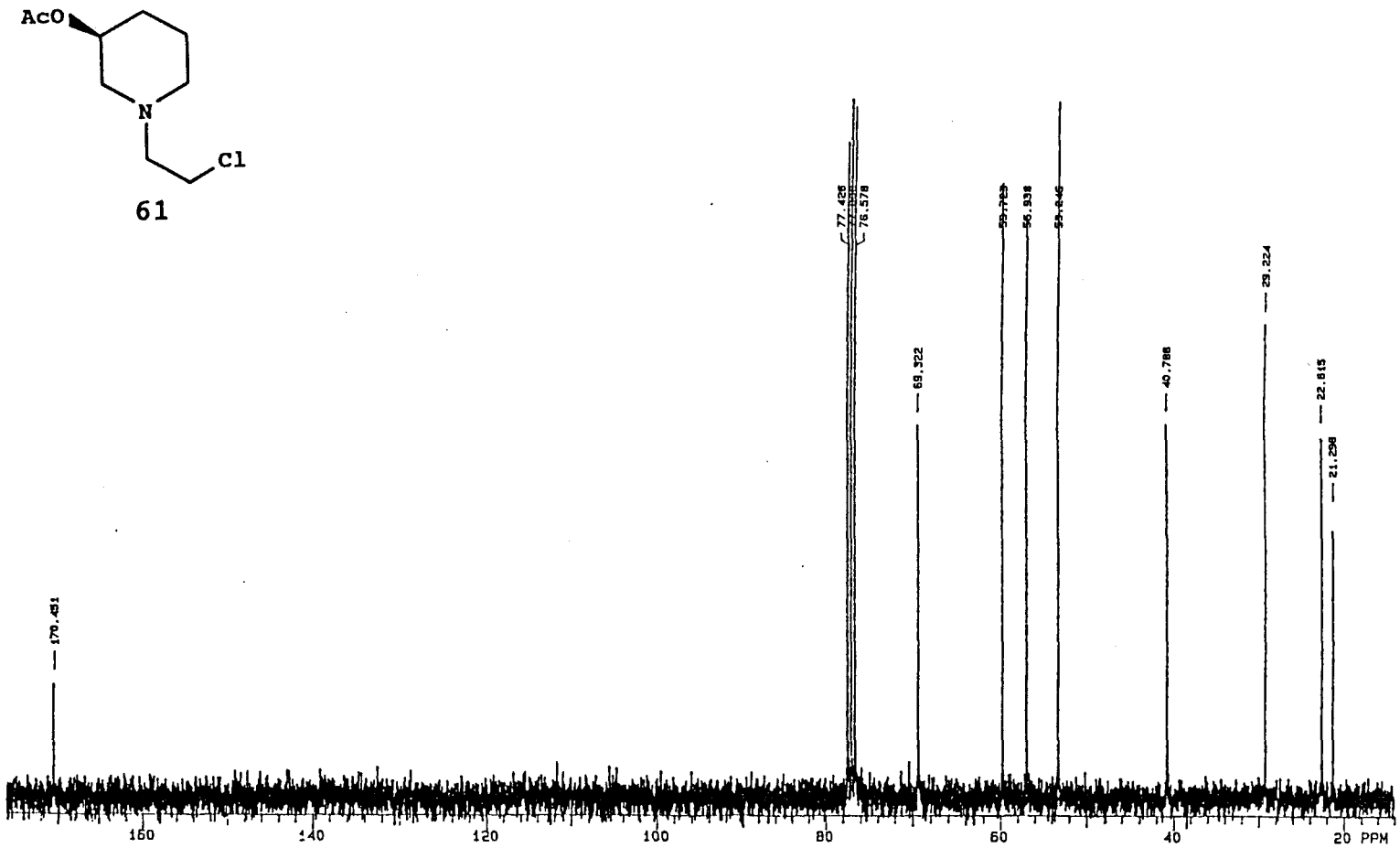
^{13}C NMR Spectrum of (R,S)-(-)-N-(2-Hydroxypropyl)-3-acetoxypiperidine (**60**)



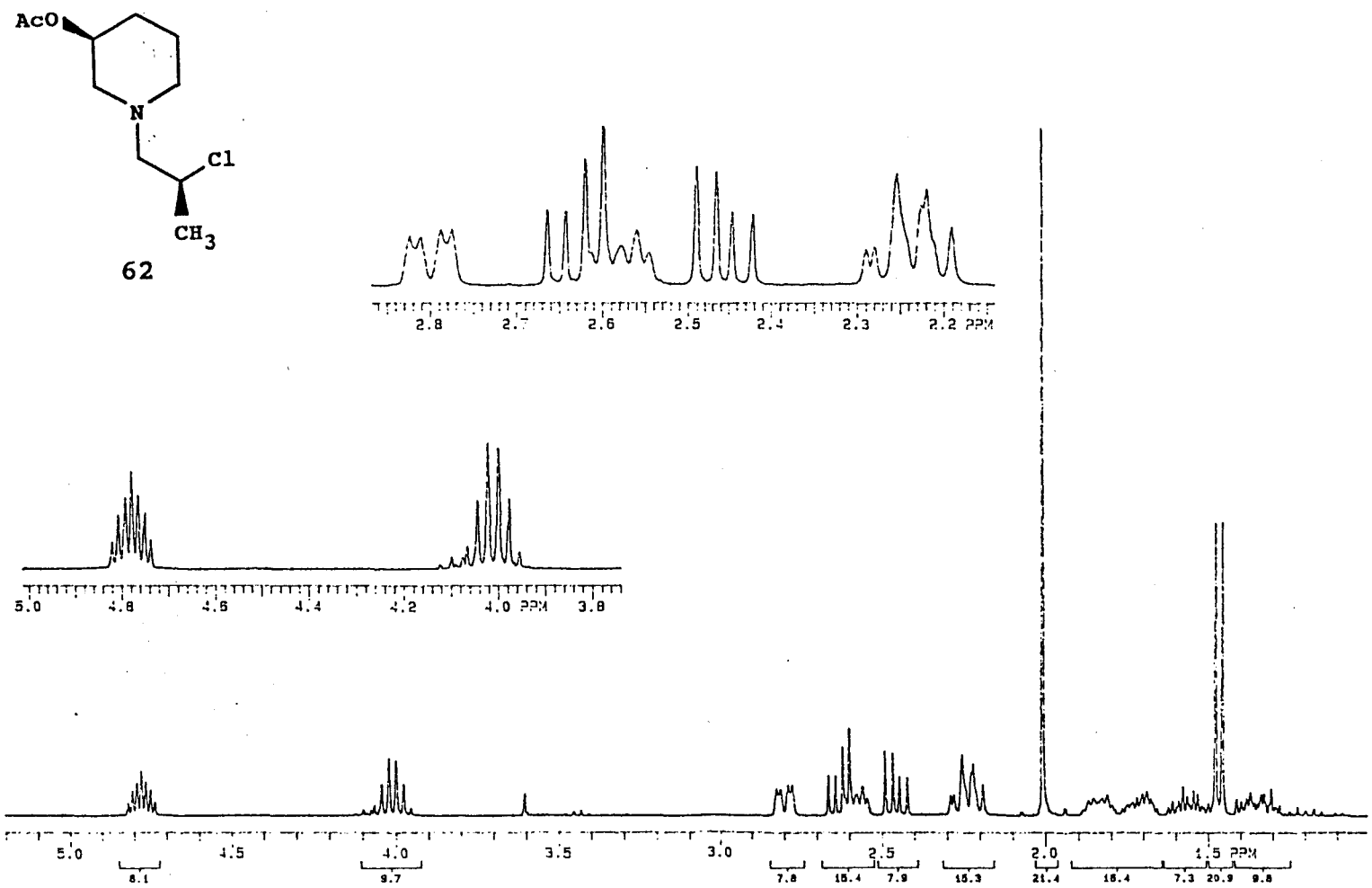
61



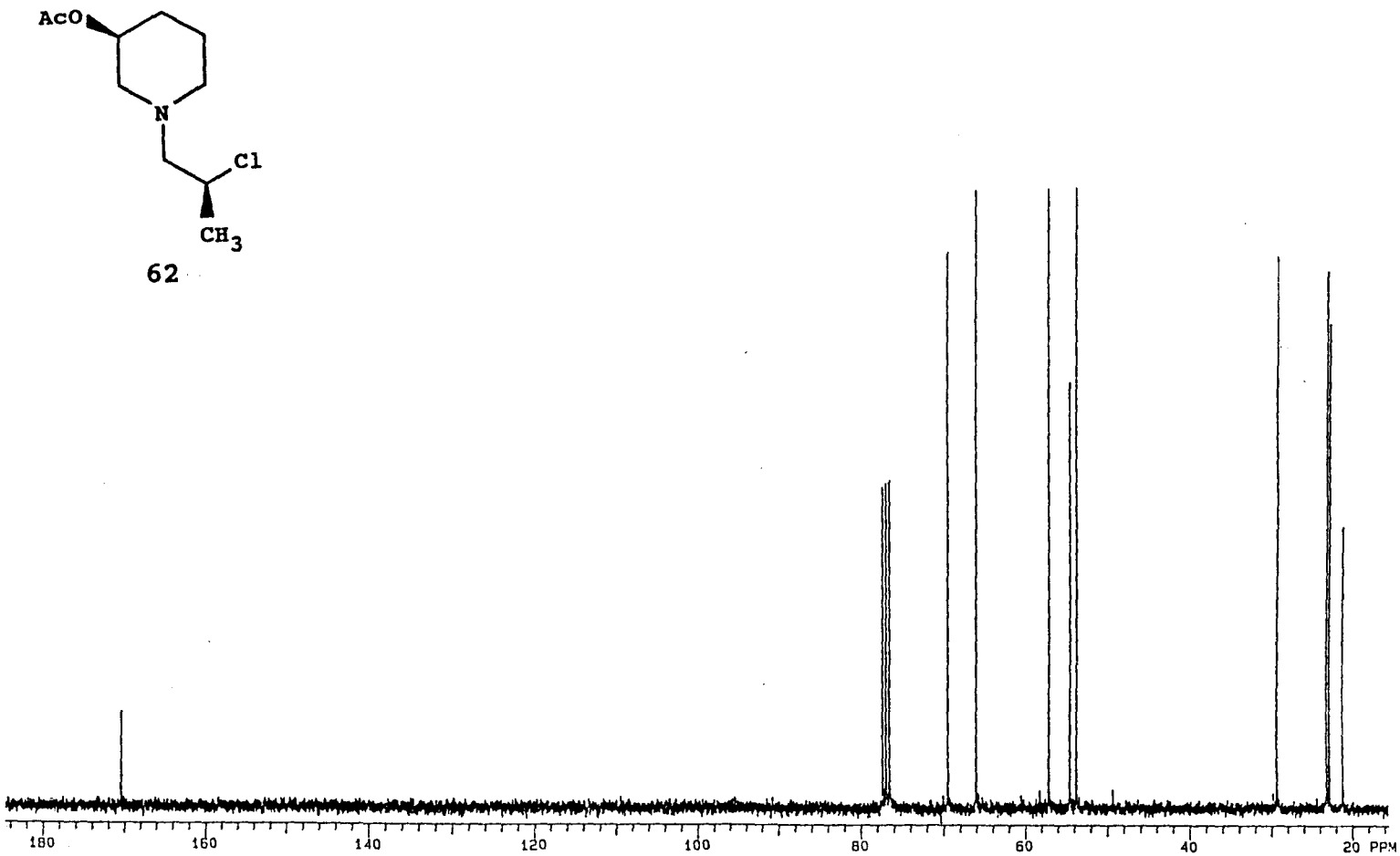
^{1}H NMR Spectrum of (S)-(-)-N-(2-Chloroethyl)-3-acetoxypiperidine (**61**)



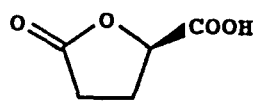
^{13}C NMR Spectrum of (S)-(-)-N-(2-Chloroethyl)-3-acetoxypiperidine (**61**)



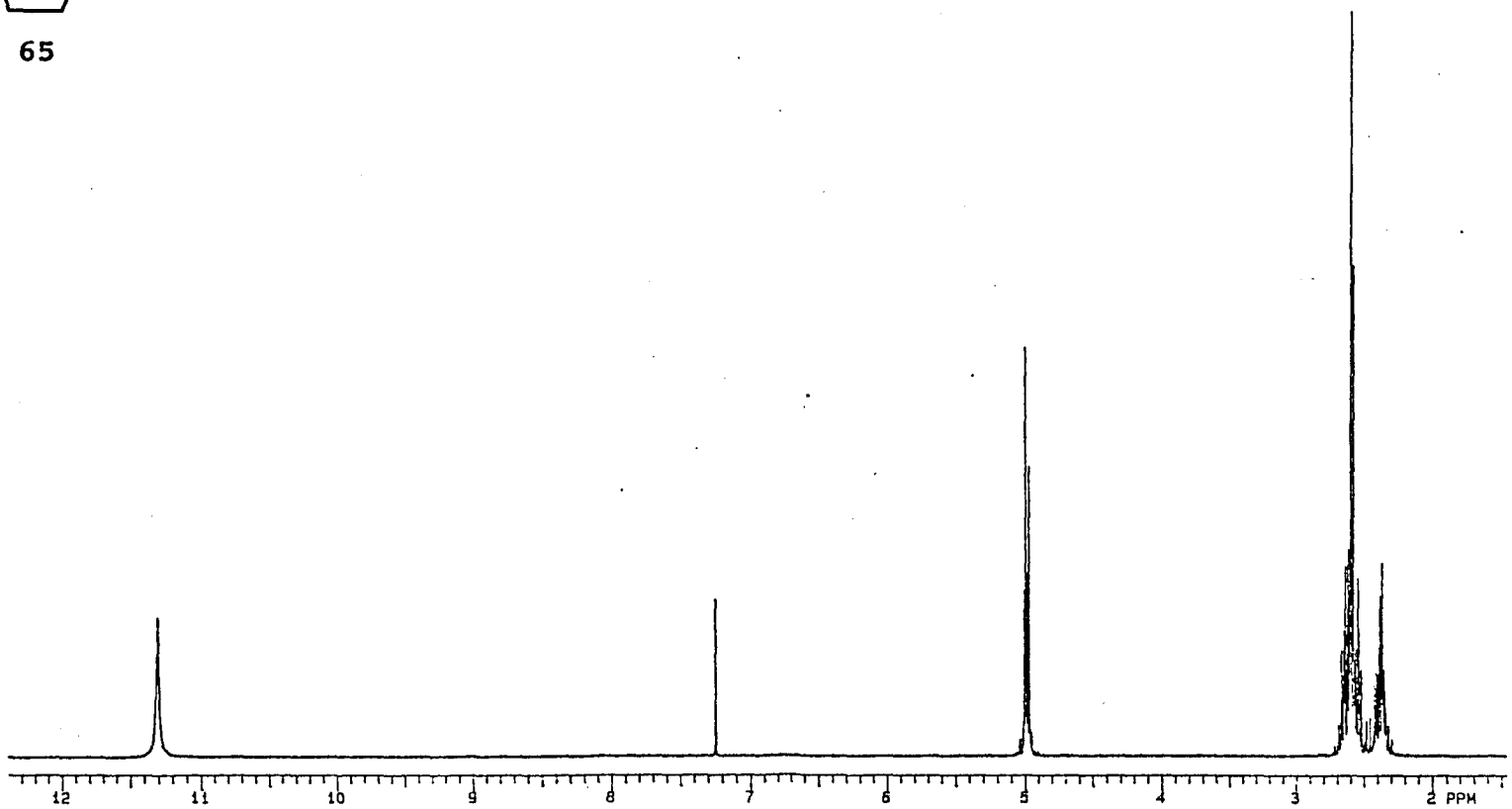
^{1}H NMR Spectrum of (S,S)-(-)-N-(2-Chloroethyl)-3-acetoxypiperidine (**62**)



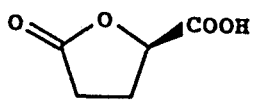
^{13}C NMR Spectrum of (S,S)-(-)-N-(2-Chloroethyl)-3-acetoxypiperidine (**62**)



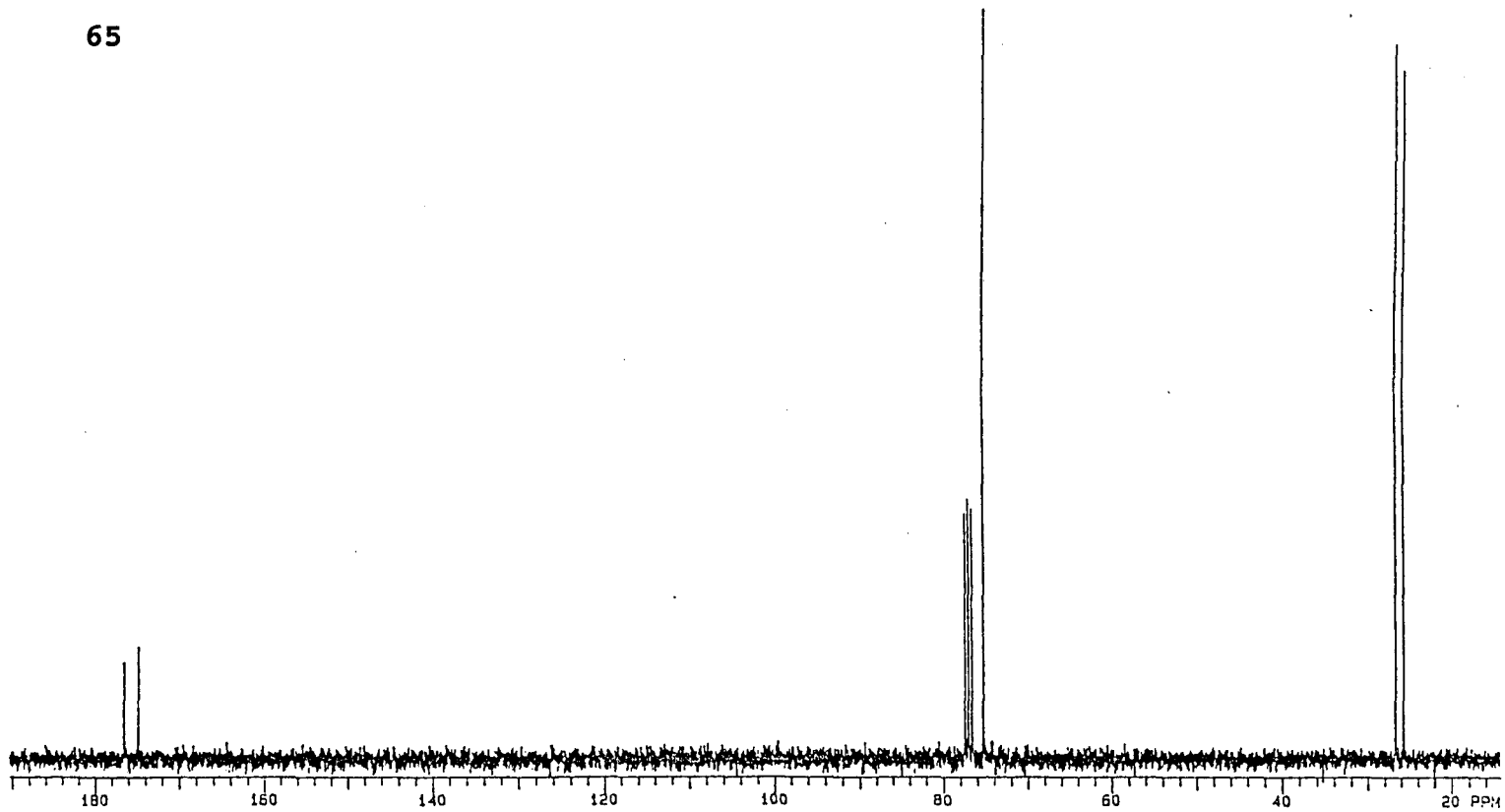
65



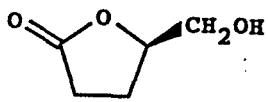
^{1}H NMR Spectrum of (R)-(-)-5-Oxo-2-tetrahydrofurancarboxylic acid (**65**)



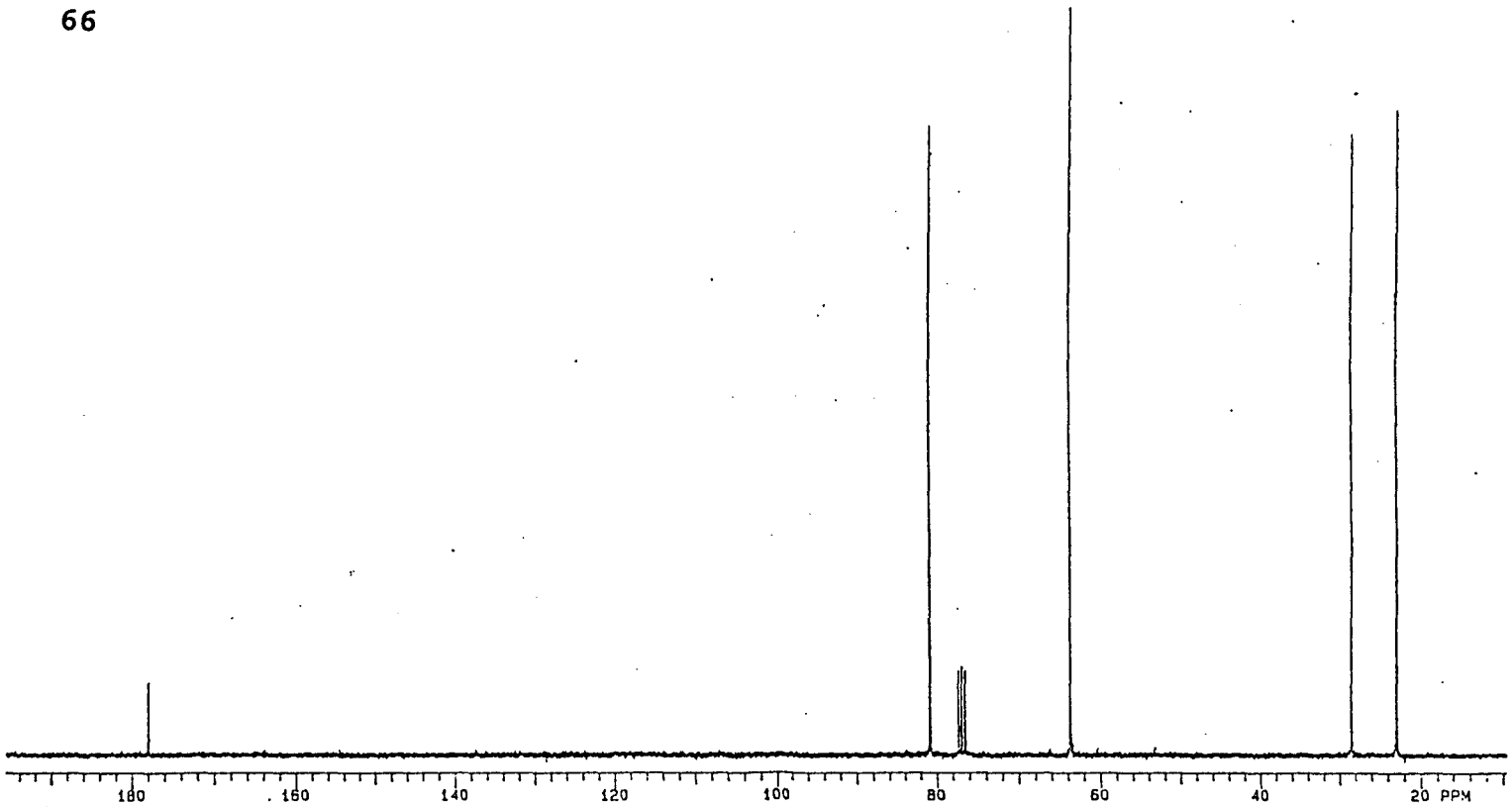
65



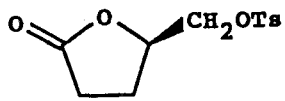
^{13}C NMR Spectrum of (R)-(-)-5-Oxo-2-tetrahydrofurancarboxylic acid (**65**)



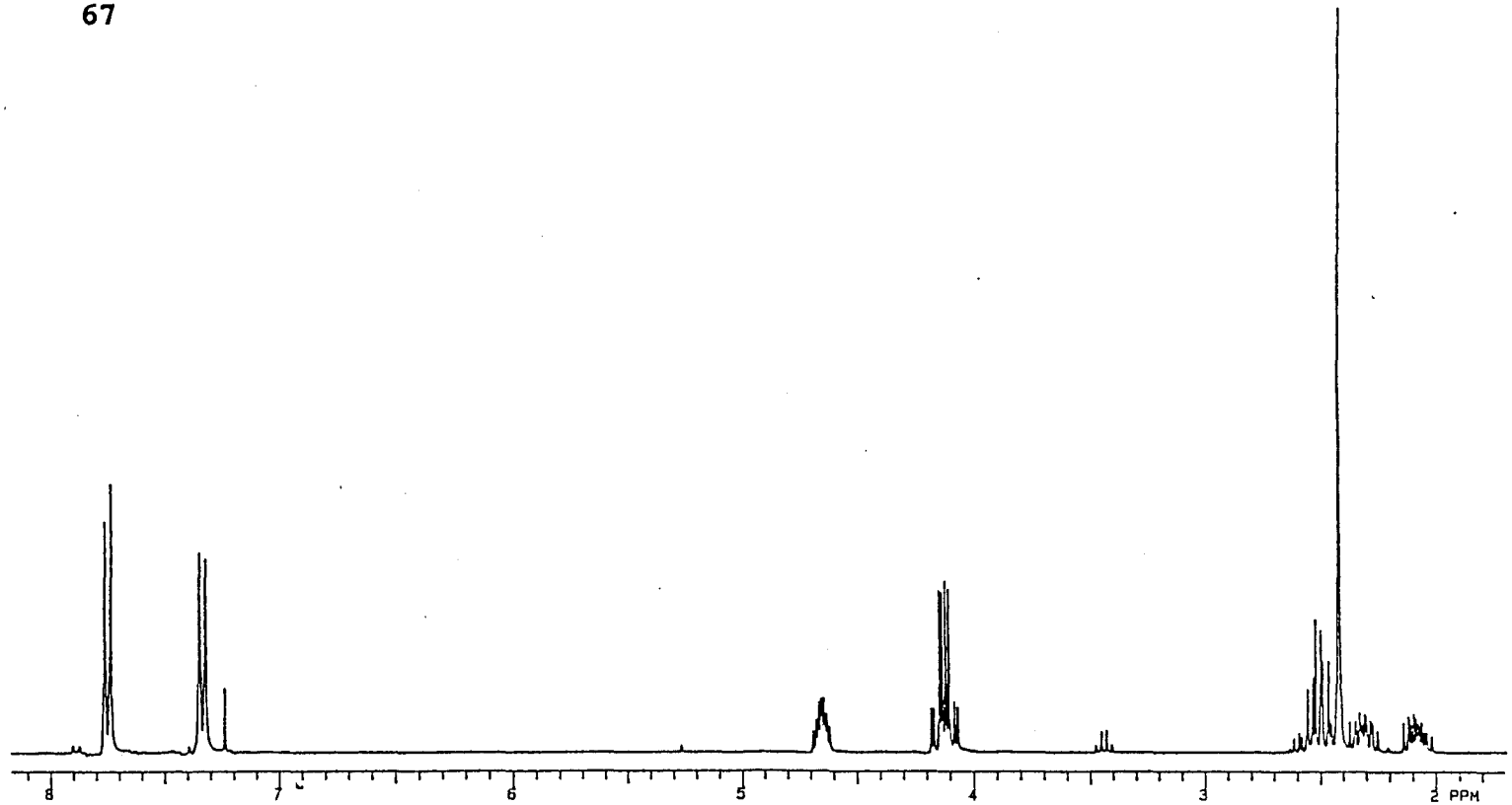
66



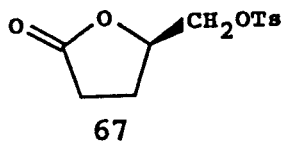
^{13}C NMR Spectrum of (R)-(-)-γ-Hydroxymethyl-γ-butyrolactone (**66**)



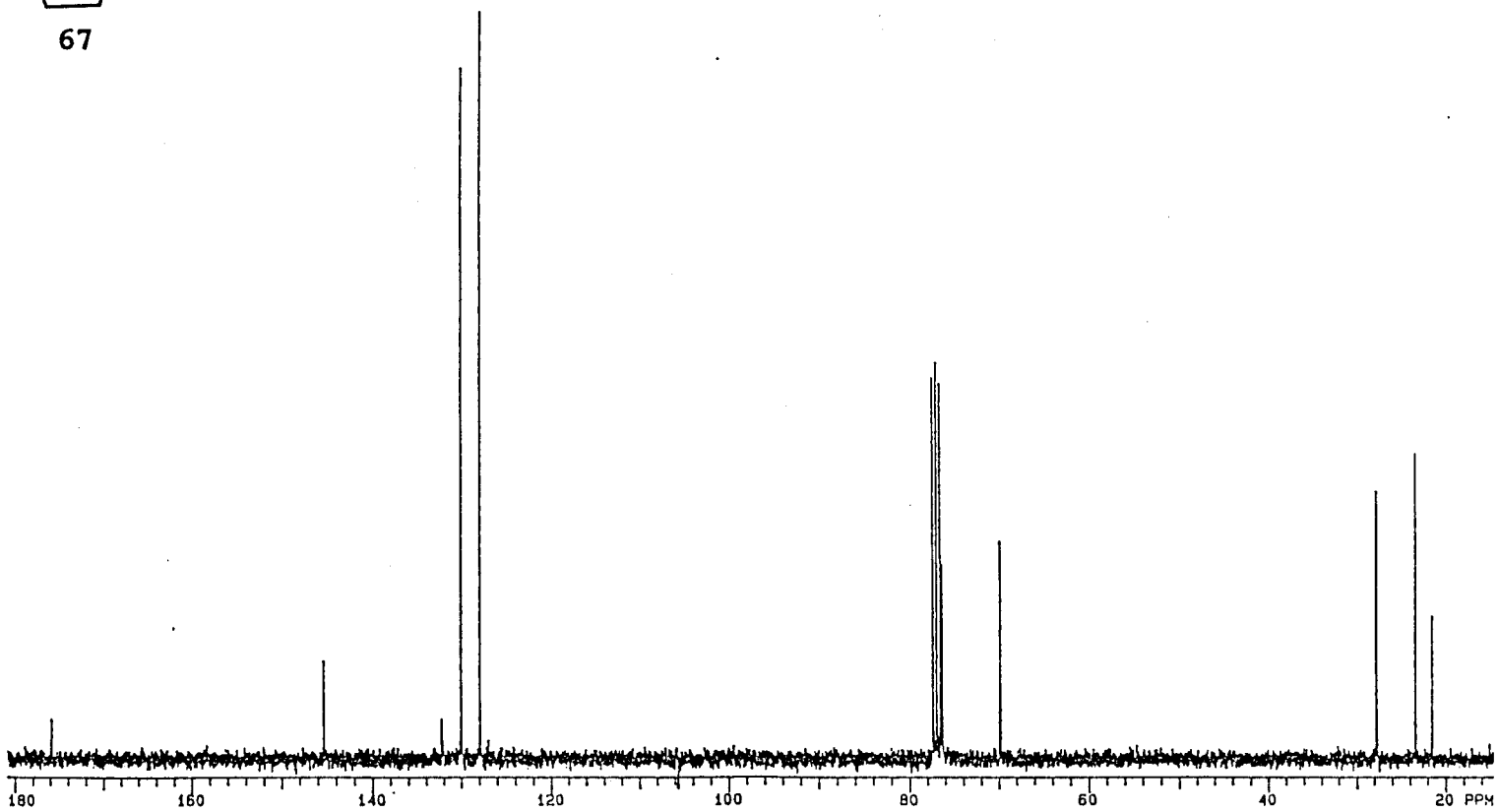
67



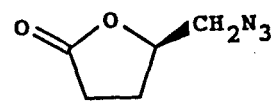
^{1}H NMR Spectrum of (R)-(-)-γ-p-Tosyloxymethyl-γ-butyrolactone (**67**)



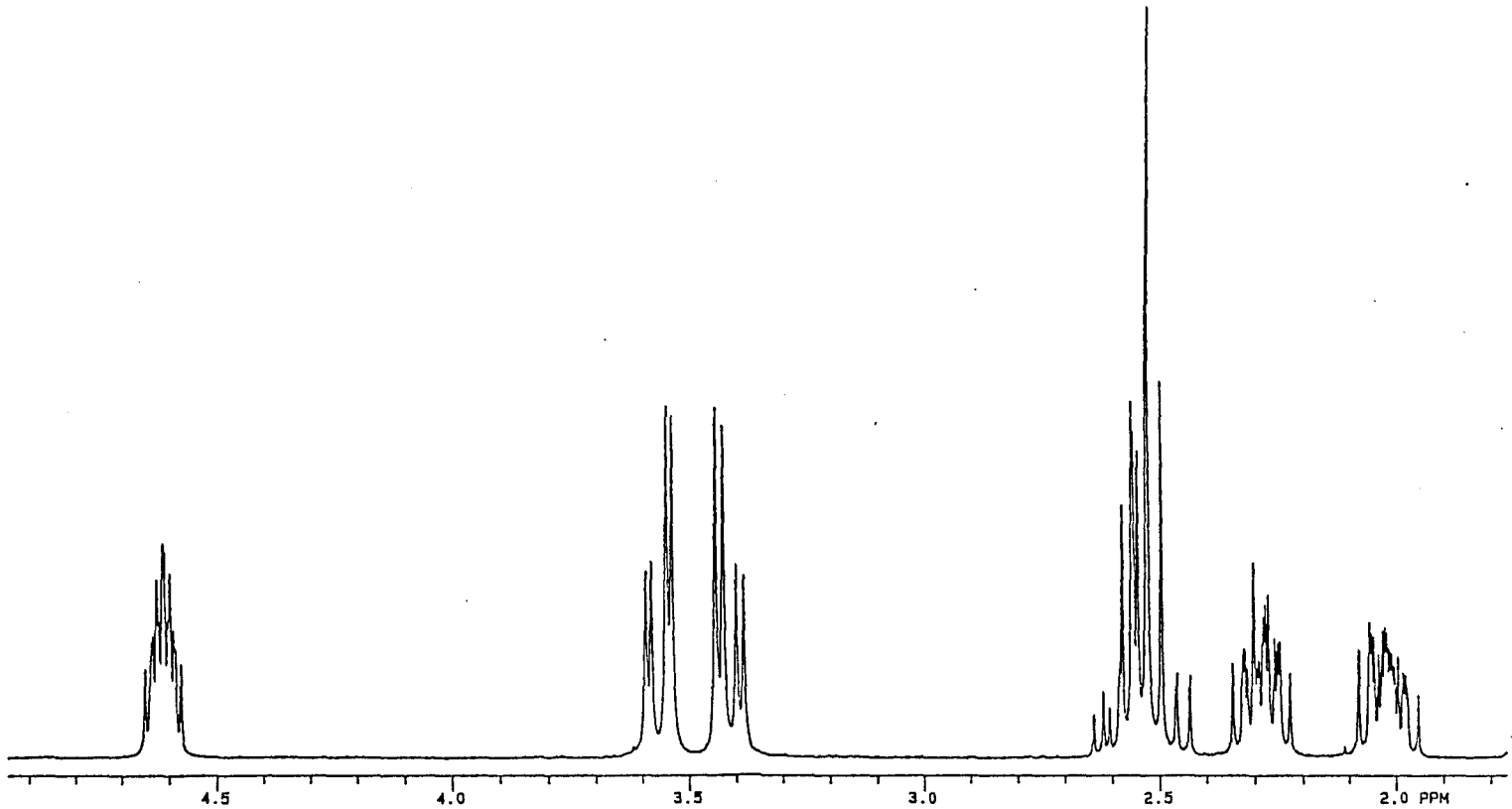
67



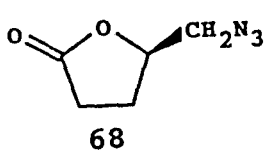
^{13}C NMR Spectrum of (R)-(-)-γ-p-Tosyloxymethyl-γ-butyrolactone (**67**)



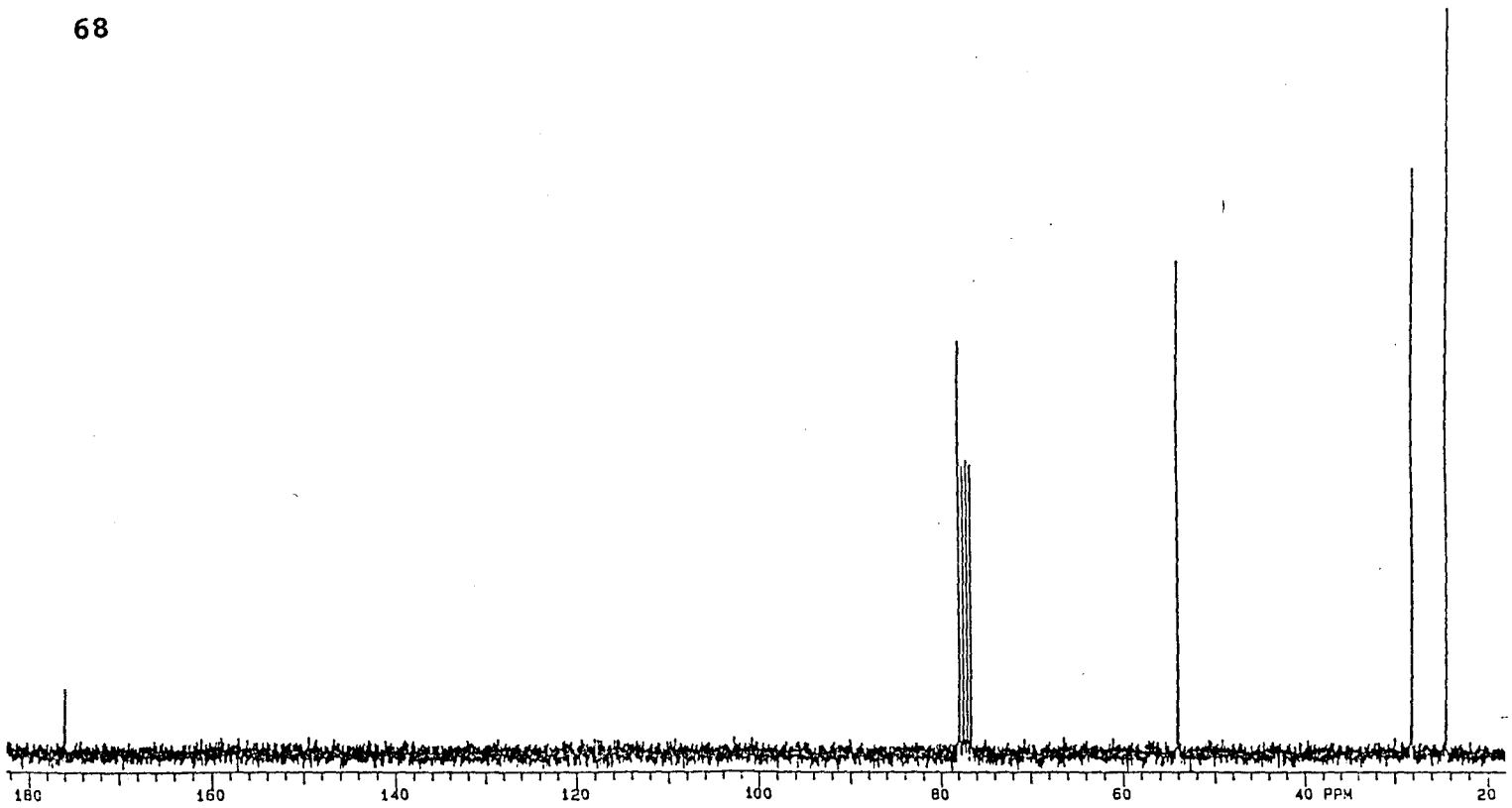
68



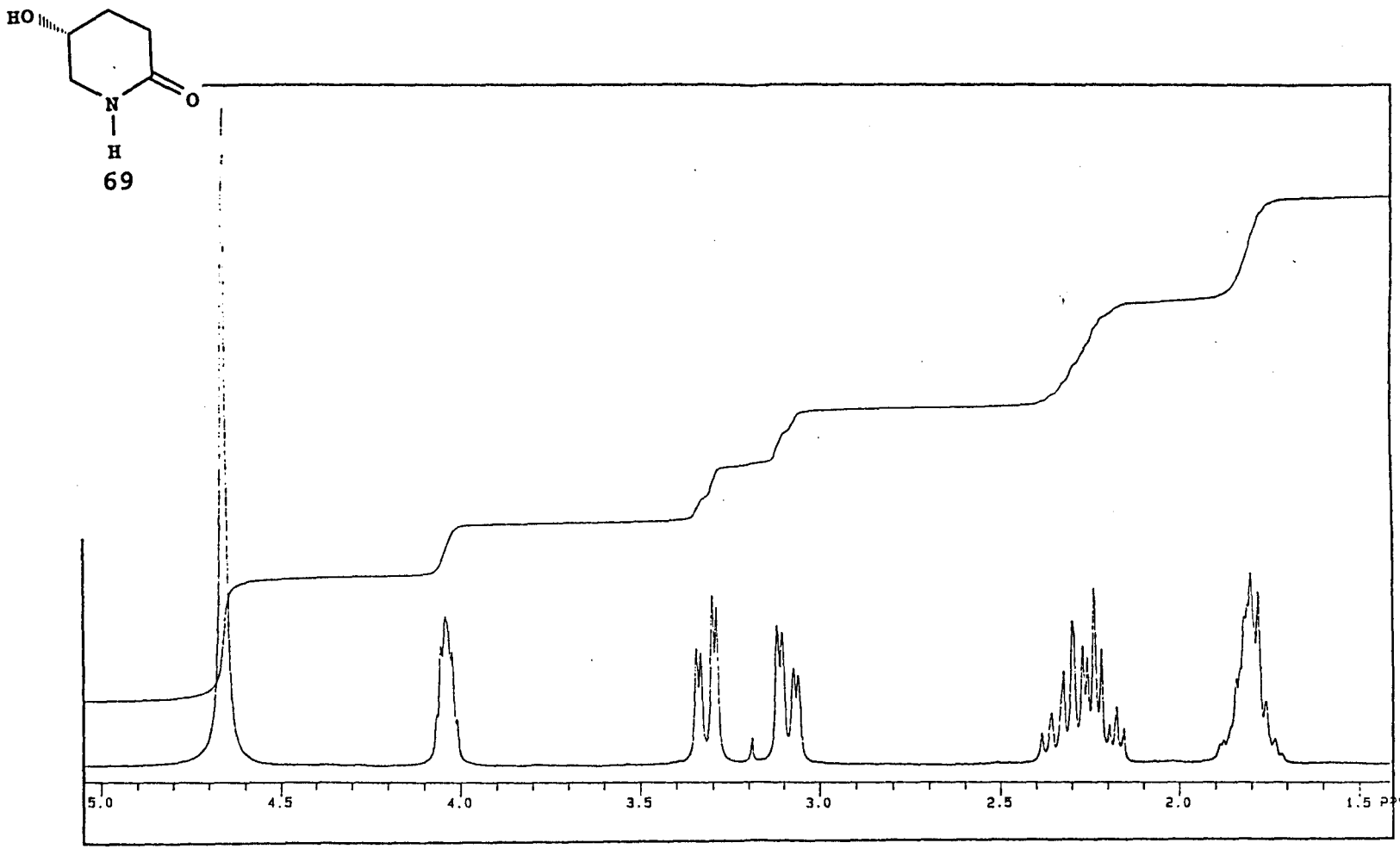
^{1}H NMR Spectrum of (R)-(-)-5-Azido-4-pentanolide (**68**)



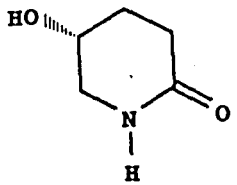
68



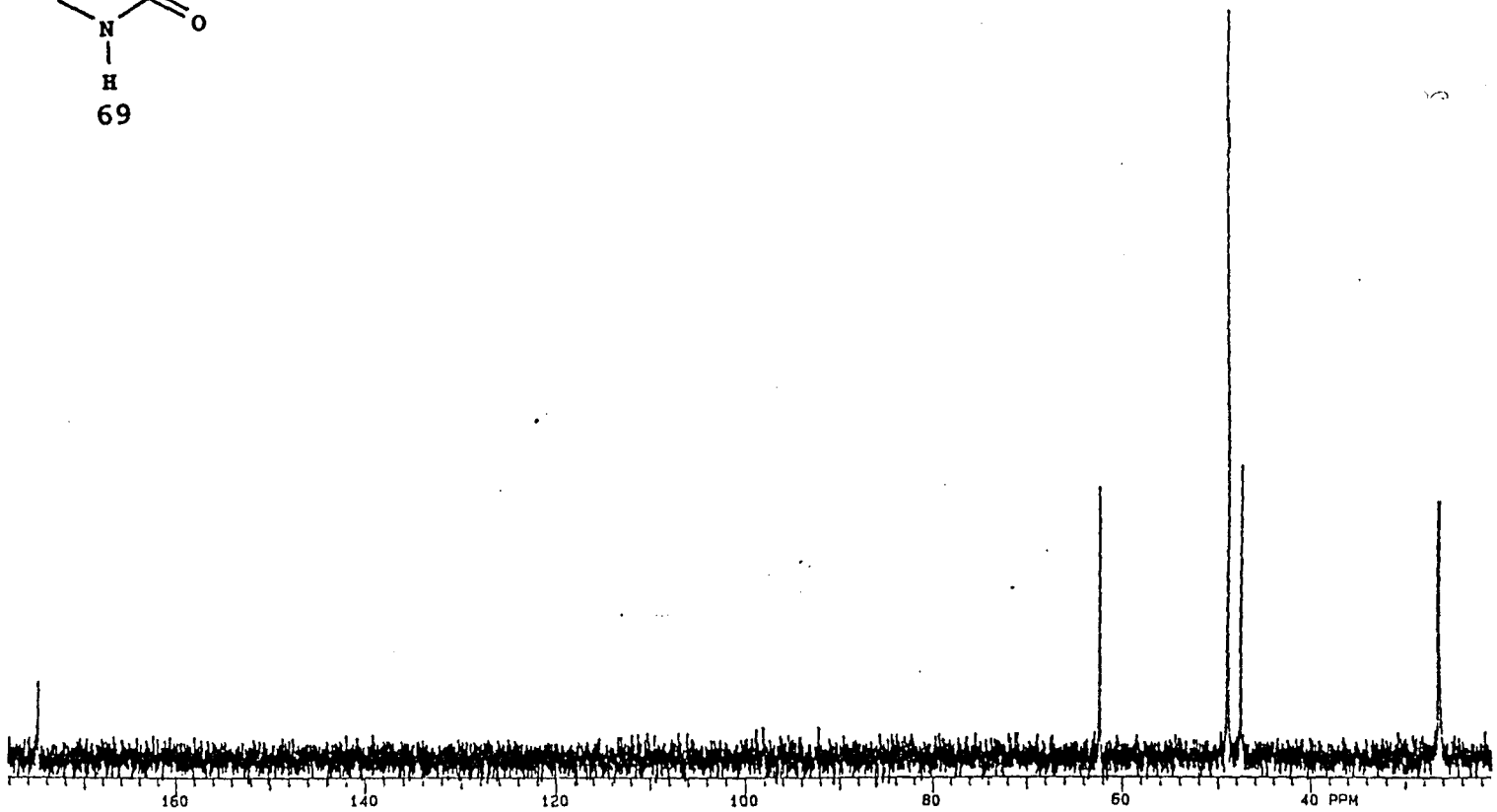
^{13}C NMR Spectrum of (R)-(-)-5-Azido-4-pentanolide (**68**)



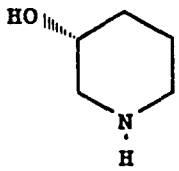
^{1}H NMR Spectrum of (R)-(+)-5-Hydroxy-2-piperidinone (**69**)



69



^{13}C NMR Spectrum of (R)-(+)-5-Hydroxy-2-piperidinone (**69**)



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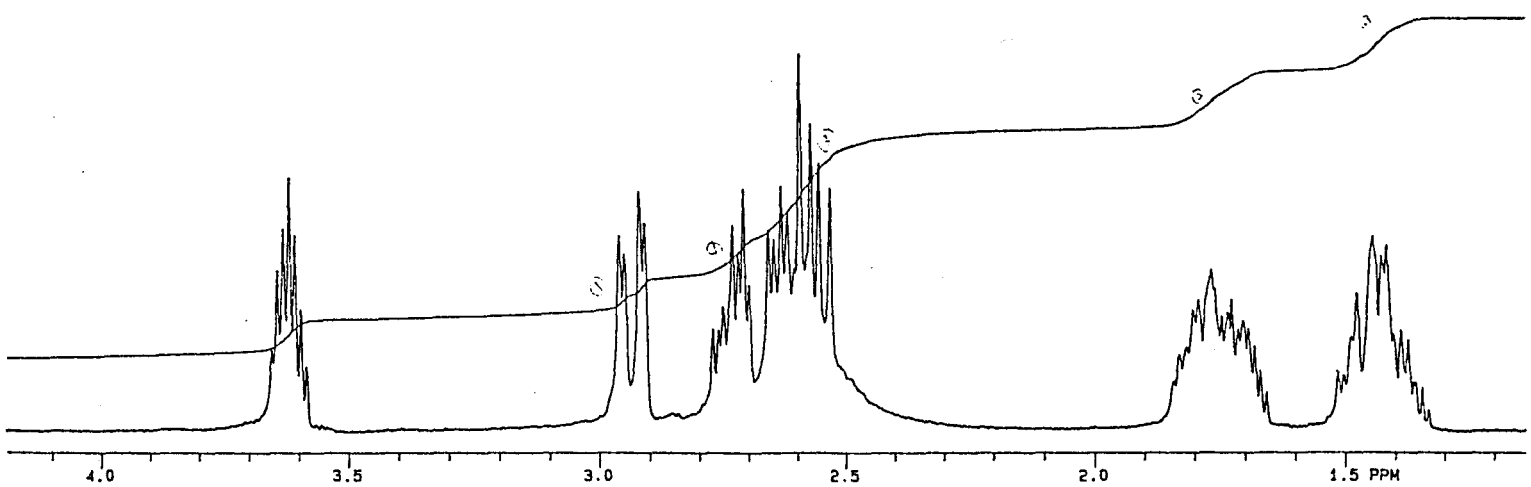
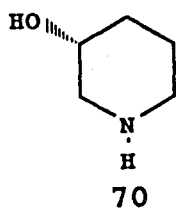
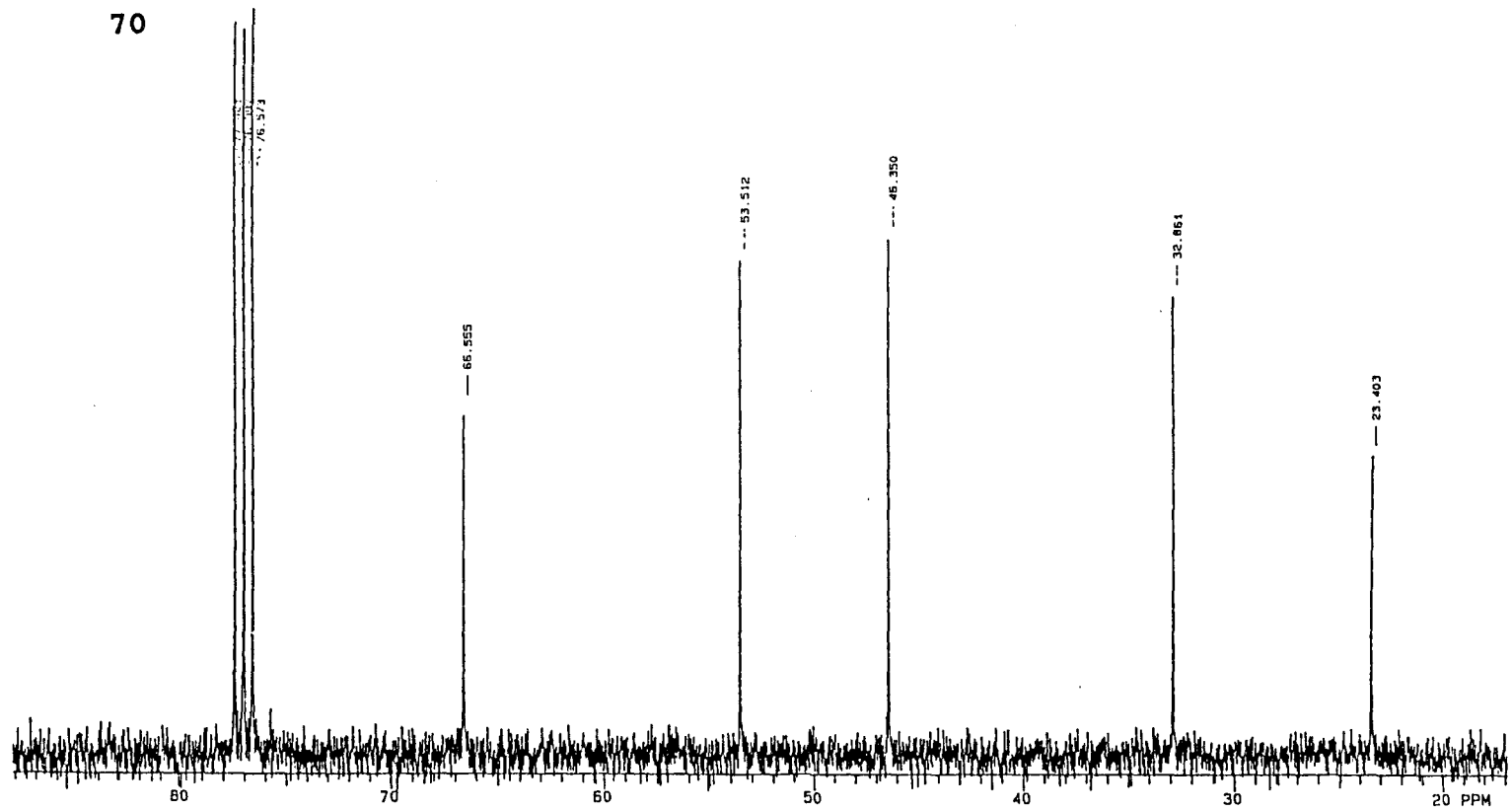


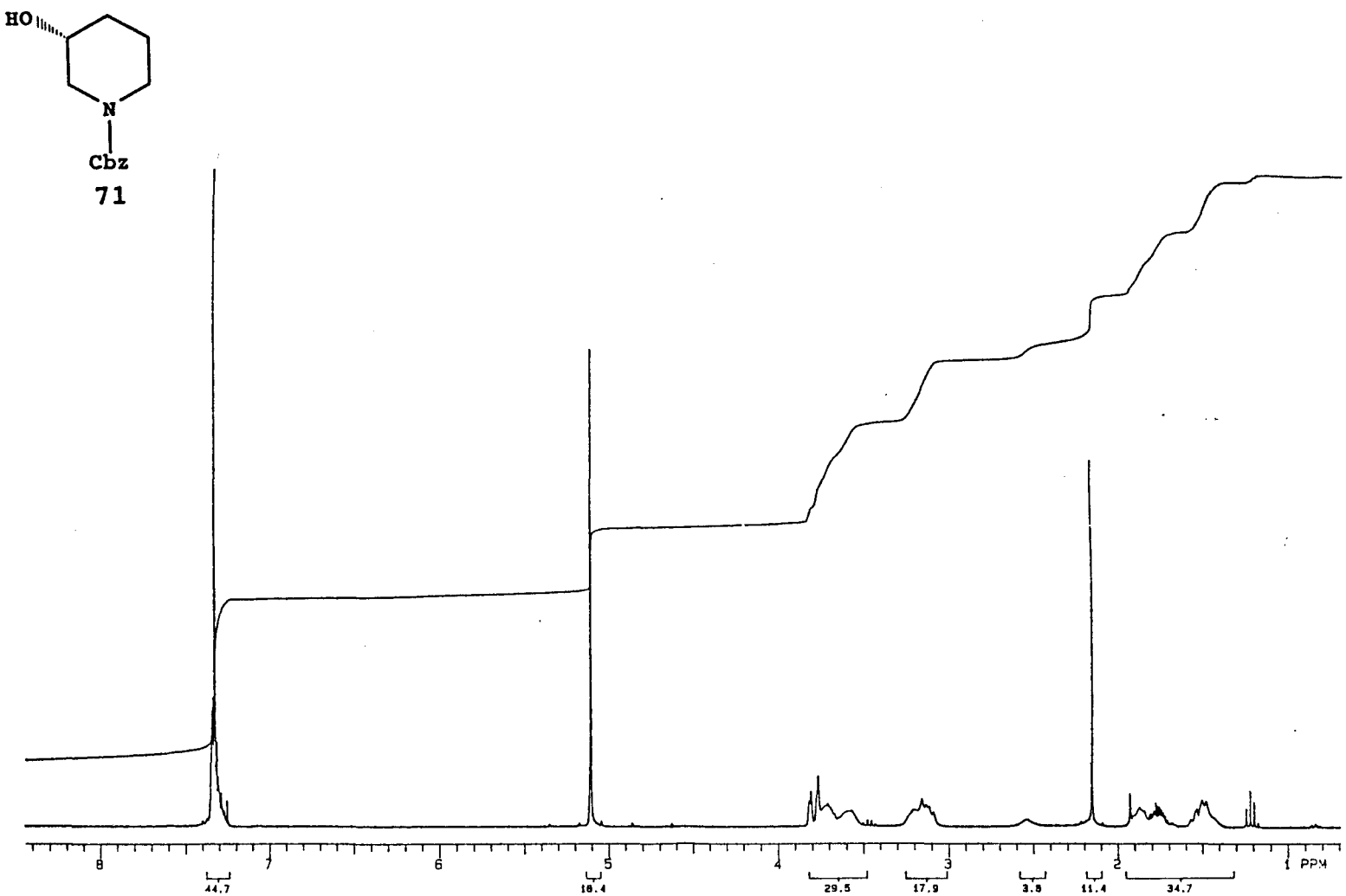
^{1}H NMR Spectrum of (R)-(+)-3-Hydroxypiperidine (**70**)



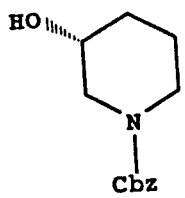
70



^{13}C NMR Spectrum of (R)-(+)-3-Hydroxypiperidine (**70**)



^{1}H NMR Spectrum of (R)-(-)-N-Cbz-3-Hydroxypiperidine (**71**)



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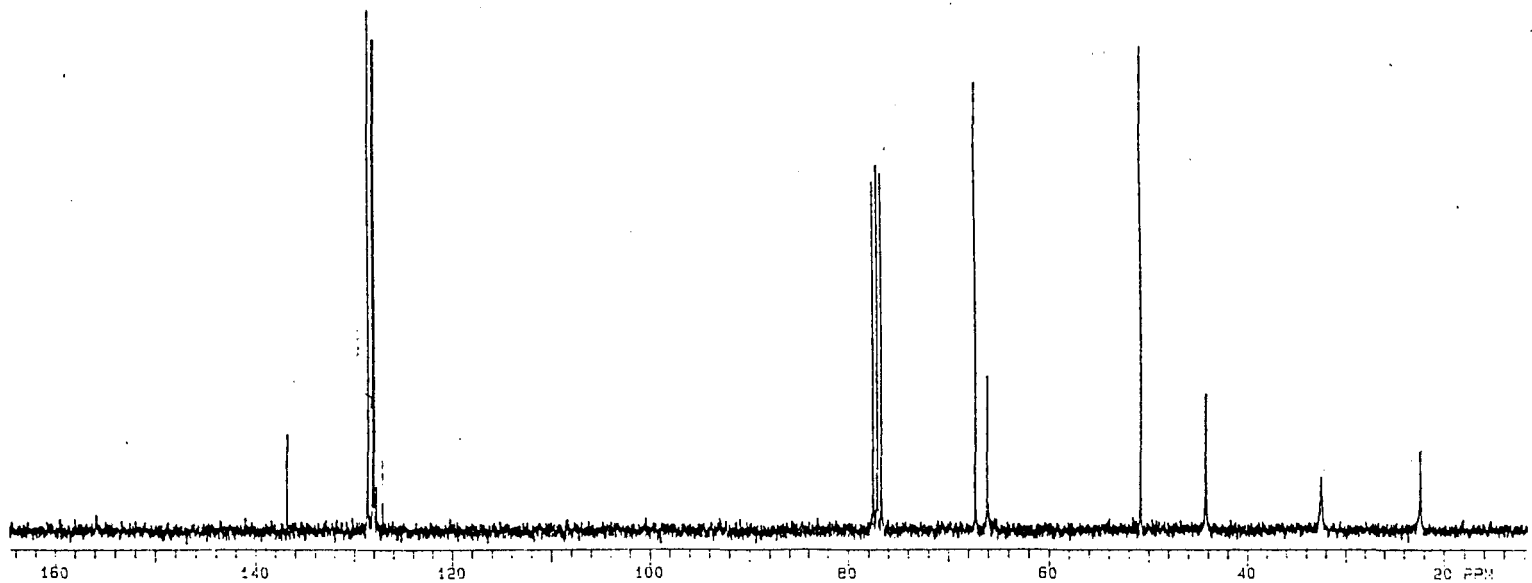
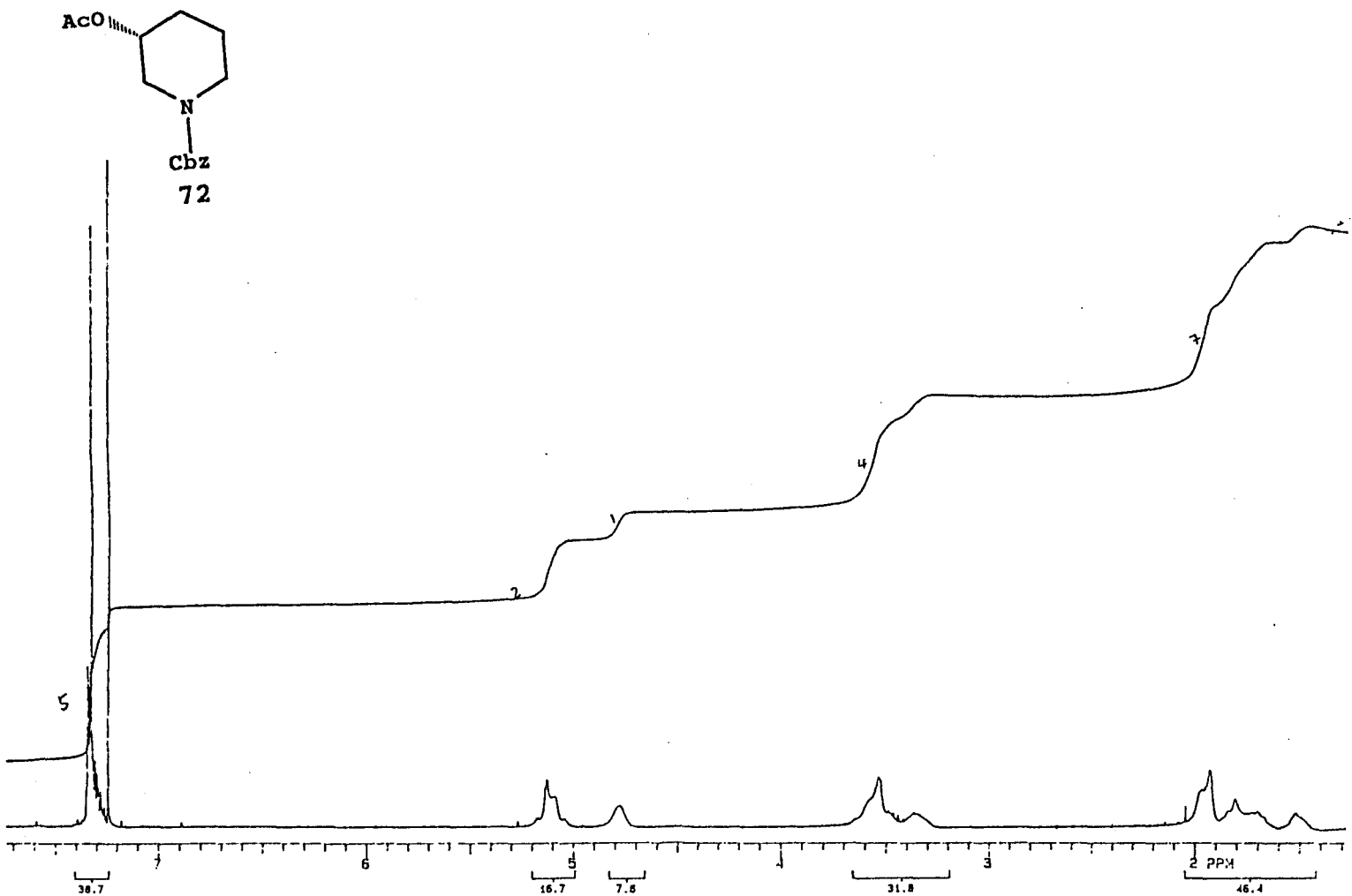
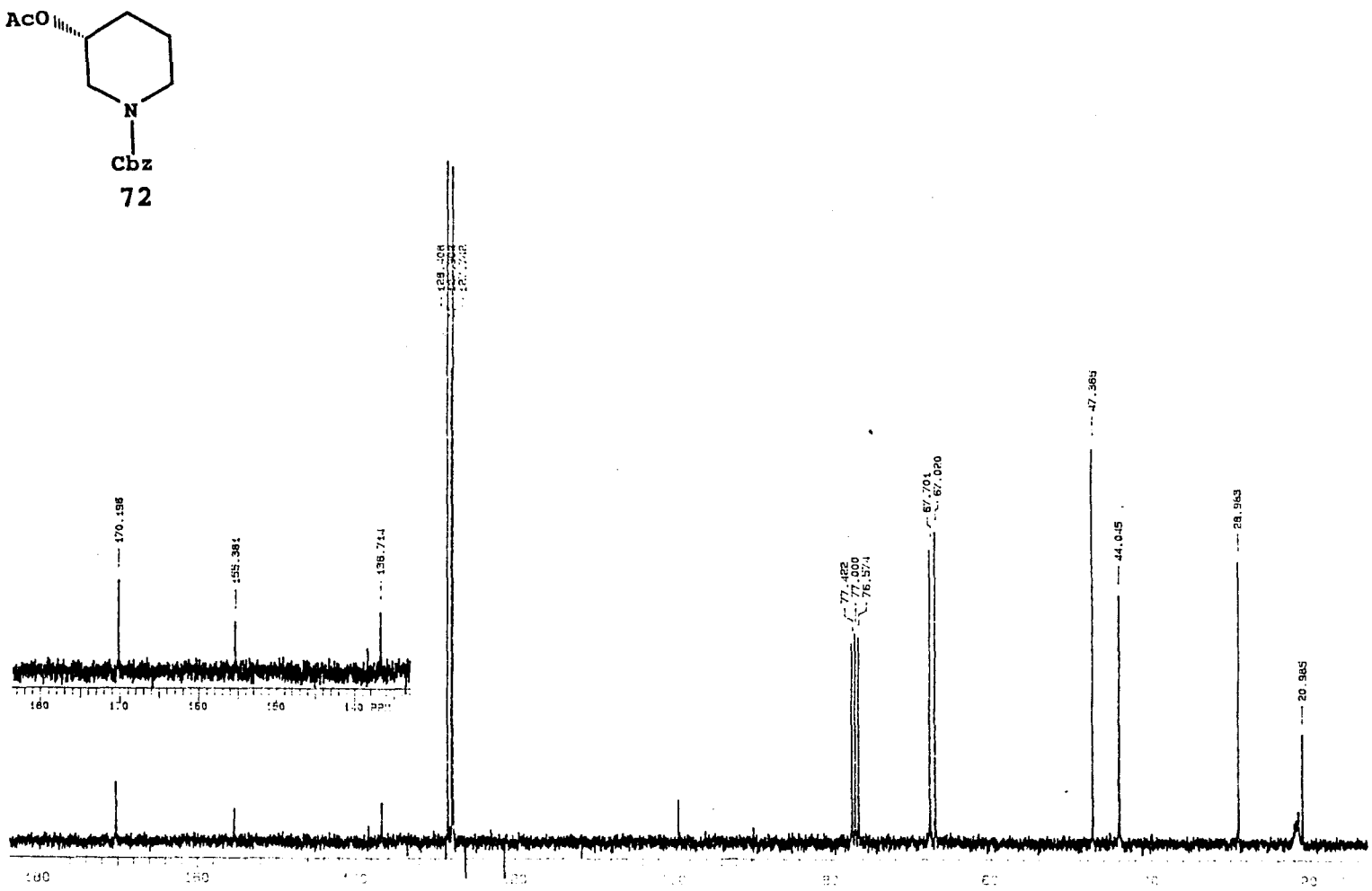


^{13}C NMR Spectrum of (R)-(-)-N-Cbz-3-Hydroxypiperidine (**71**)



^{1}H NMR Spectrum of (R)-(+)-N-Cbz-3-Acetoxypiperidine (**72**)



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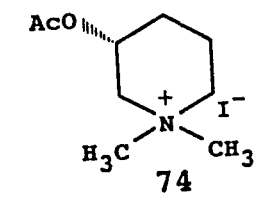

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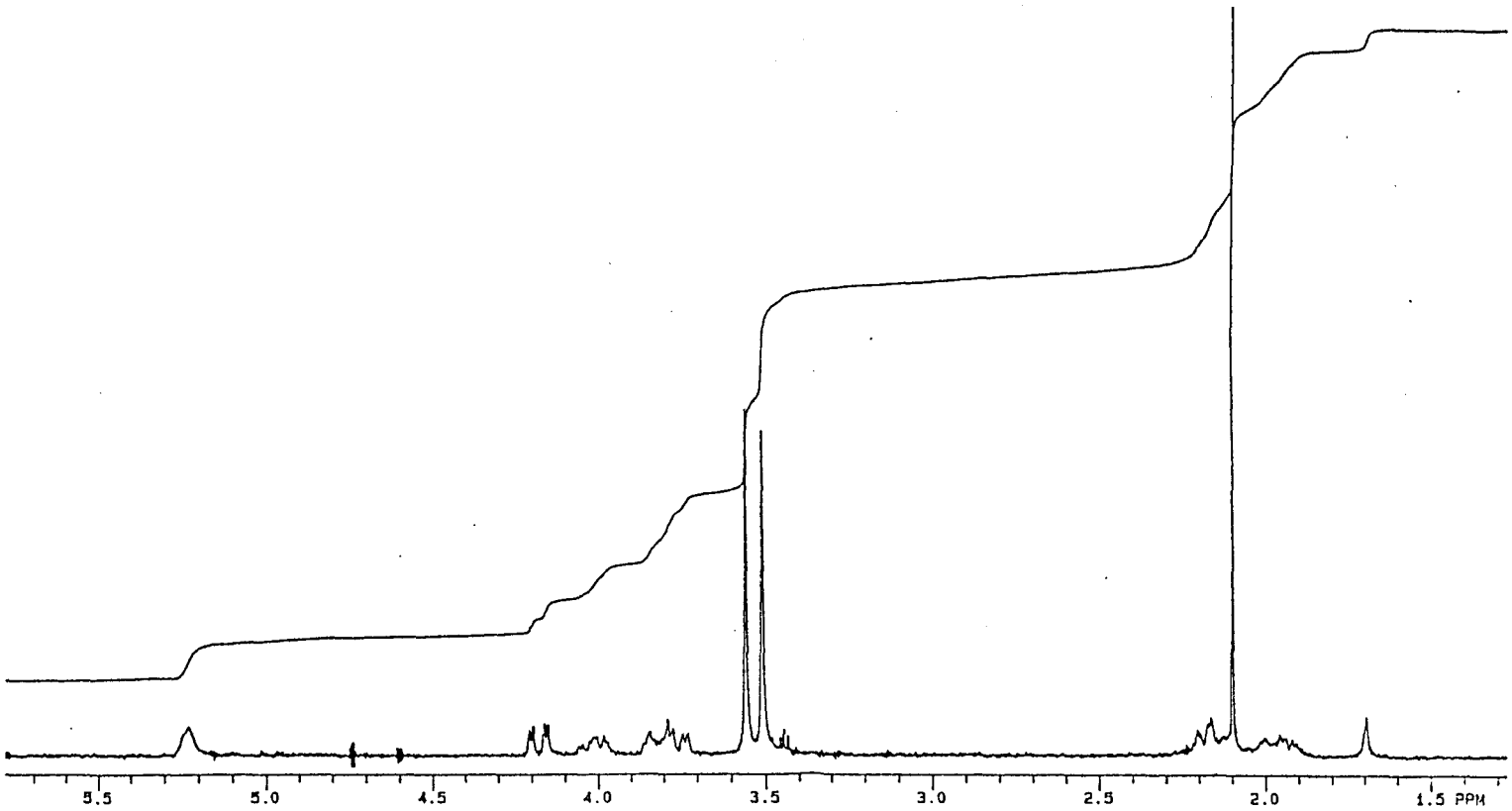
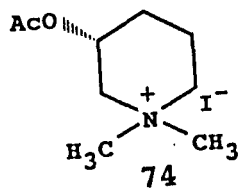


^{1}H NMR Spectrum of (S)-(-)-3-Acetoxypiperidinium Iodide (**74**)



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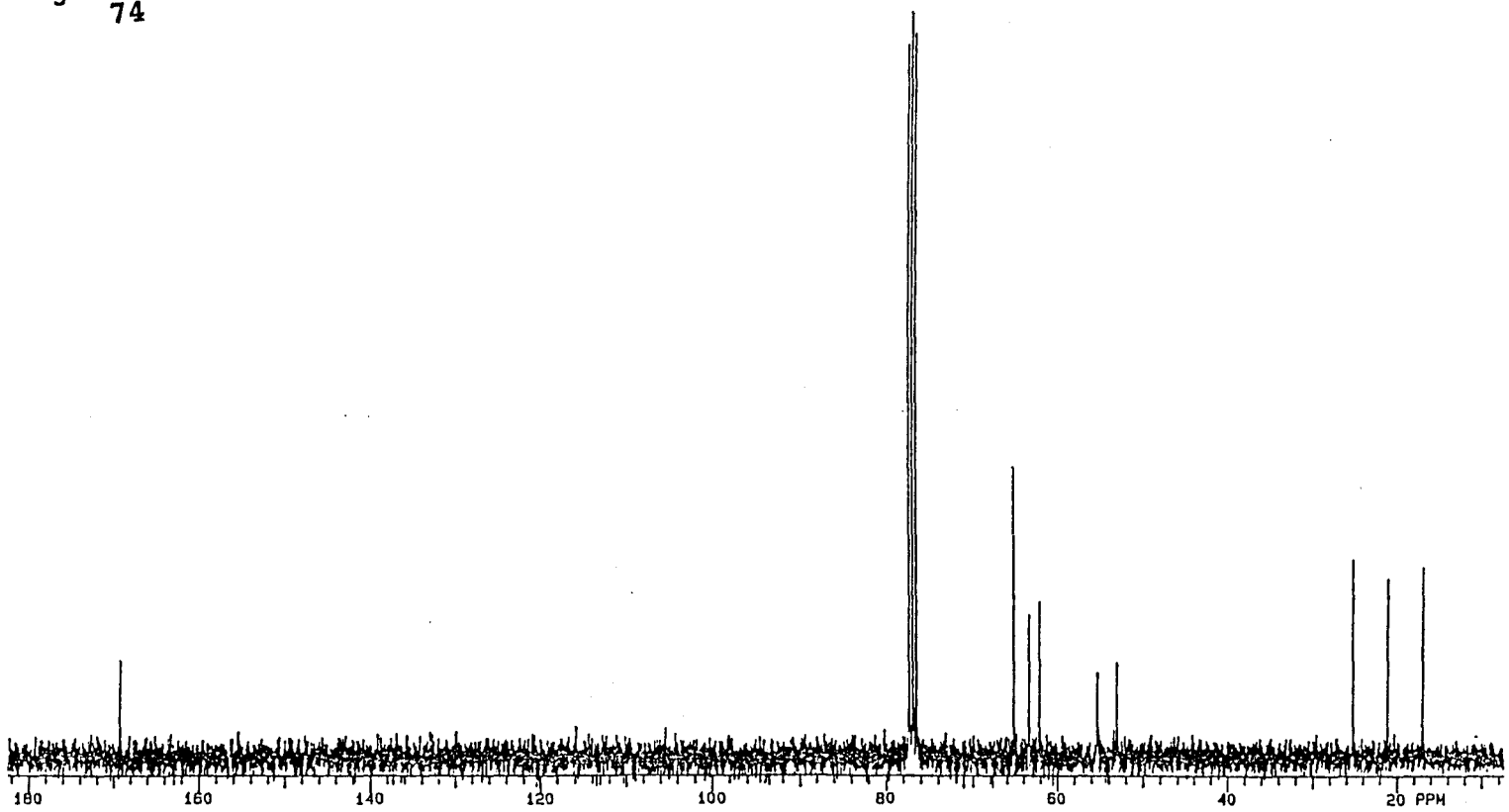
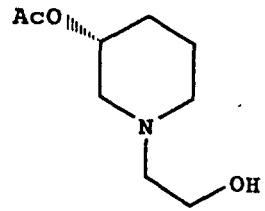


^{13}C NMR Spectrum of (S)-(-)-3-Acetoxypiperidinium Iodide (**74**)



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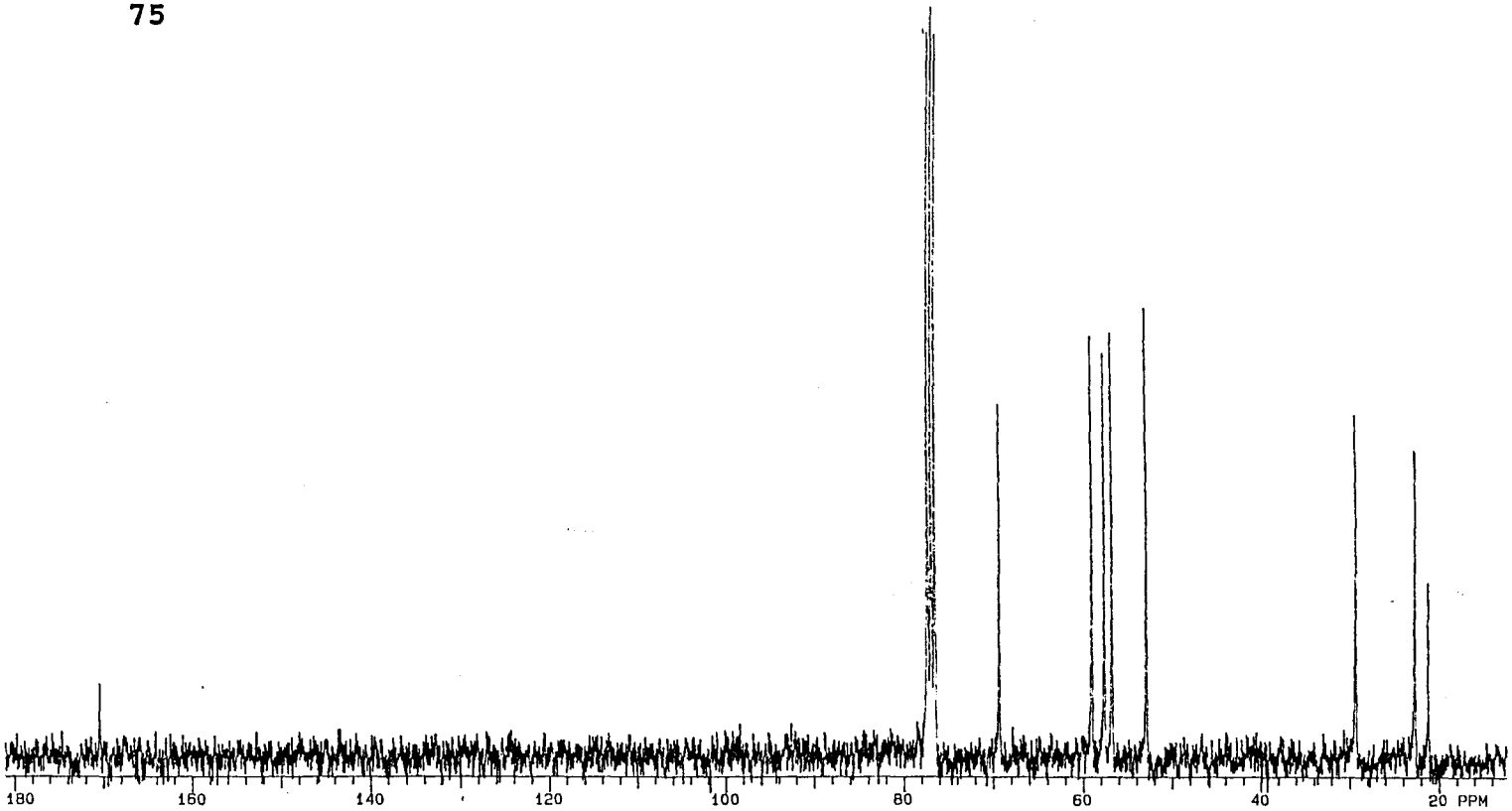
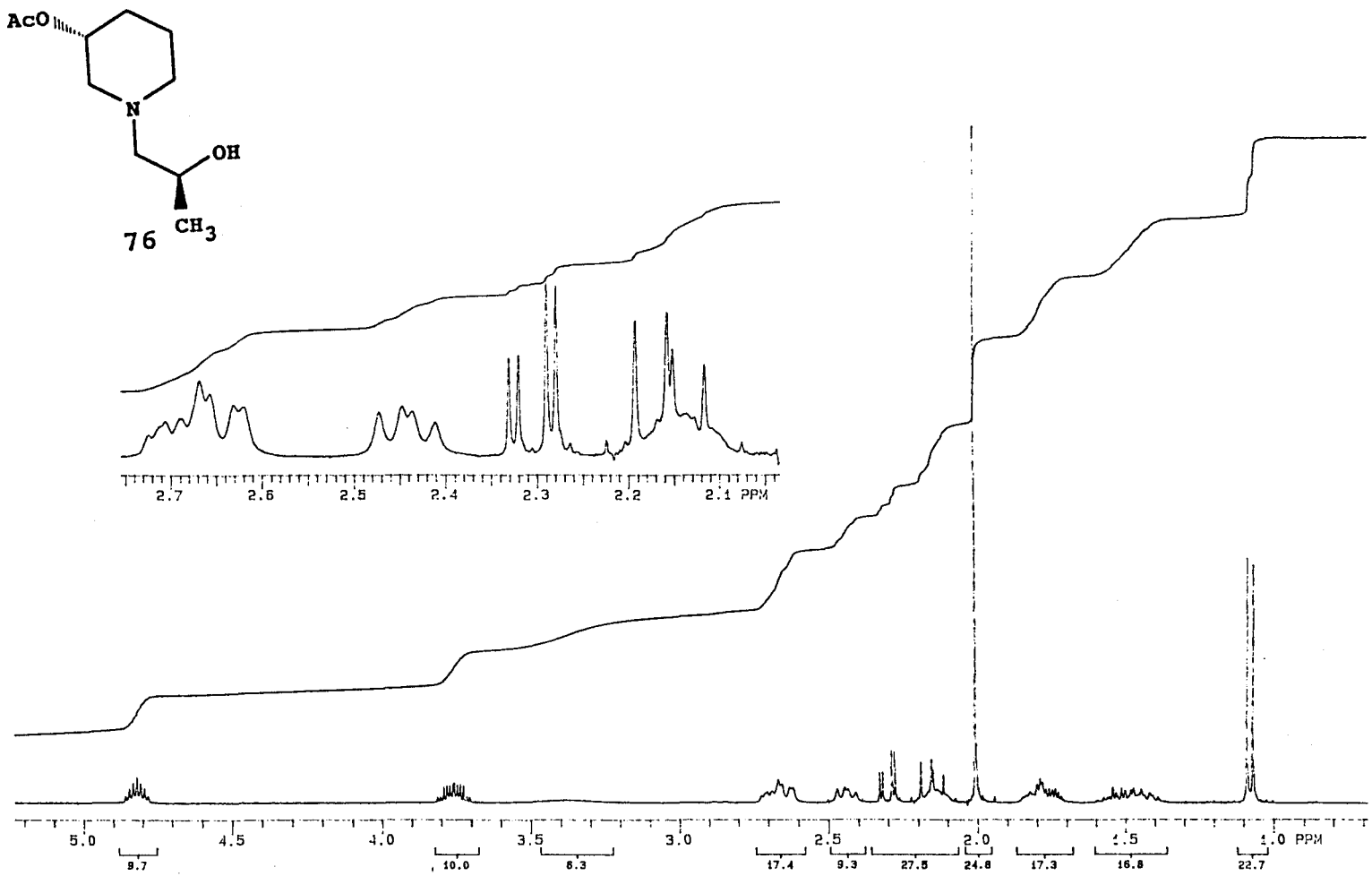
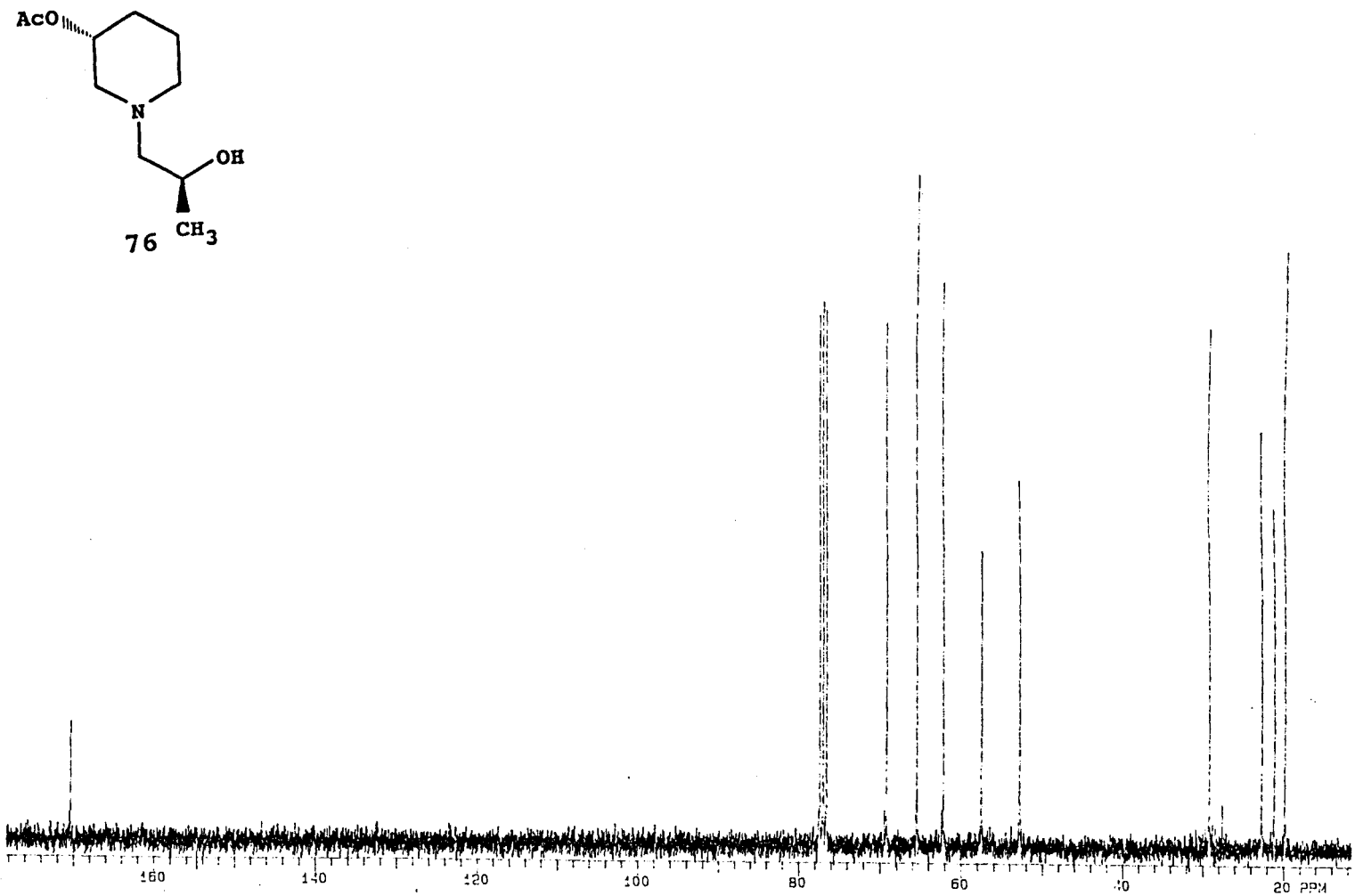


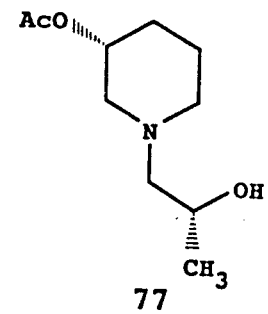
^{13}C NMR Spectrum of (R)-(+)-N-(2-Hydroxyethyl)-3-acetoxypiperidine (**75**)



^{1}H NMR Spectrum of (S,R)-(+)-N-(2-Hydroxypropyl)-3-acetoxypiperidine (**76**)



^{13}C NMR Spectrum of (S,R)-(+)-N-(2-Hydroxypropyl)-3-acetoxypiperidine (**76**)



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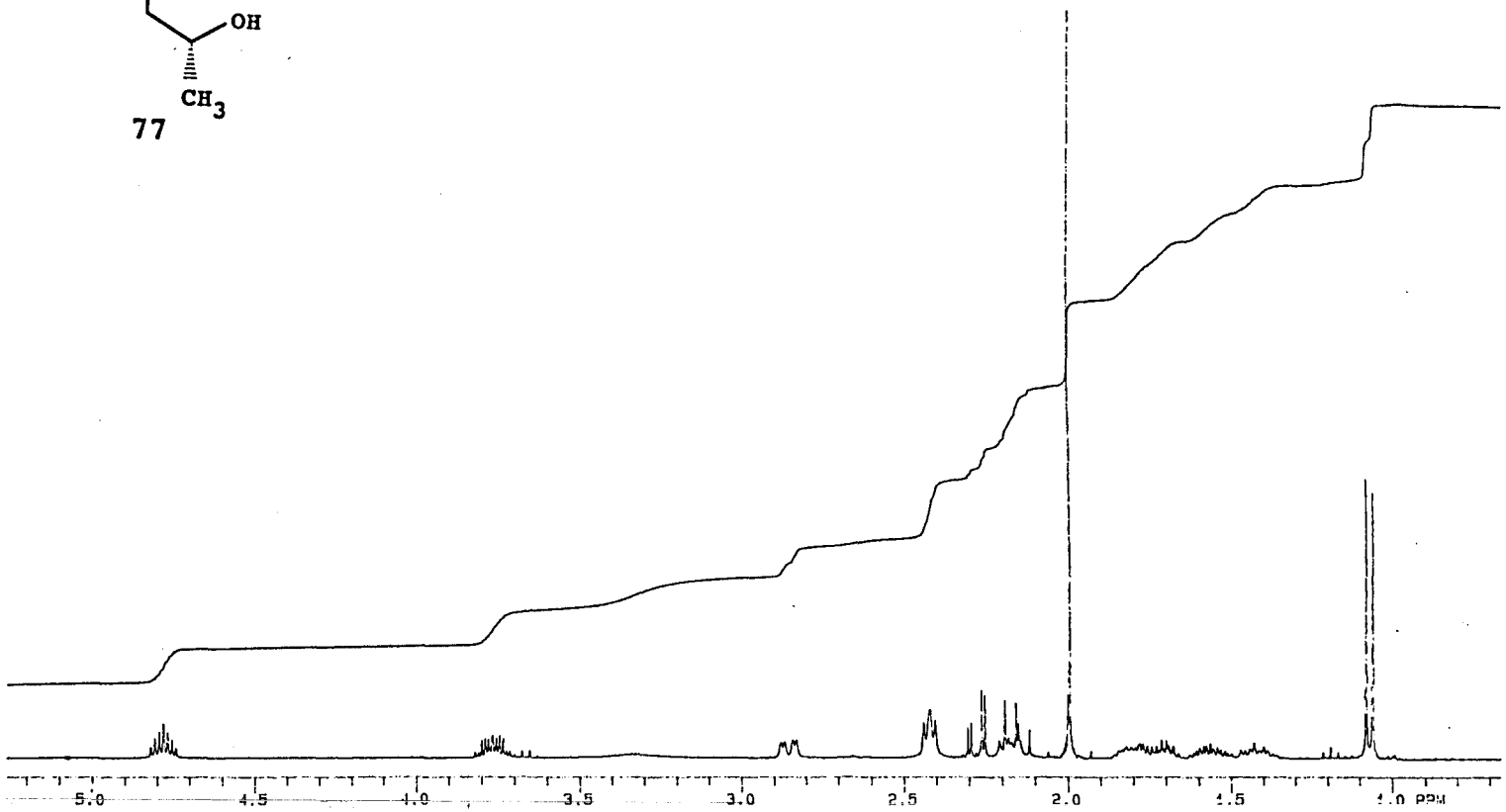
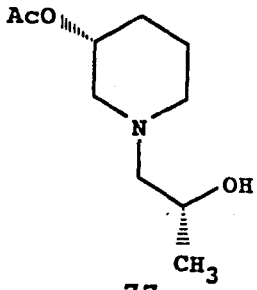


^{1}H NMR Spectrum of (R,R)-(-)-N-(2-Hydroxypropyl)-3-acetoxypiperidine (**77**)



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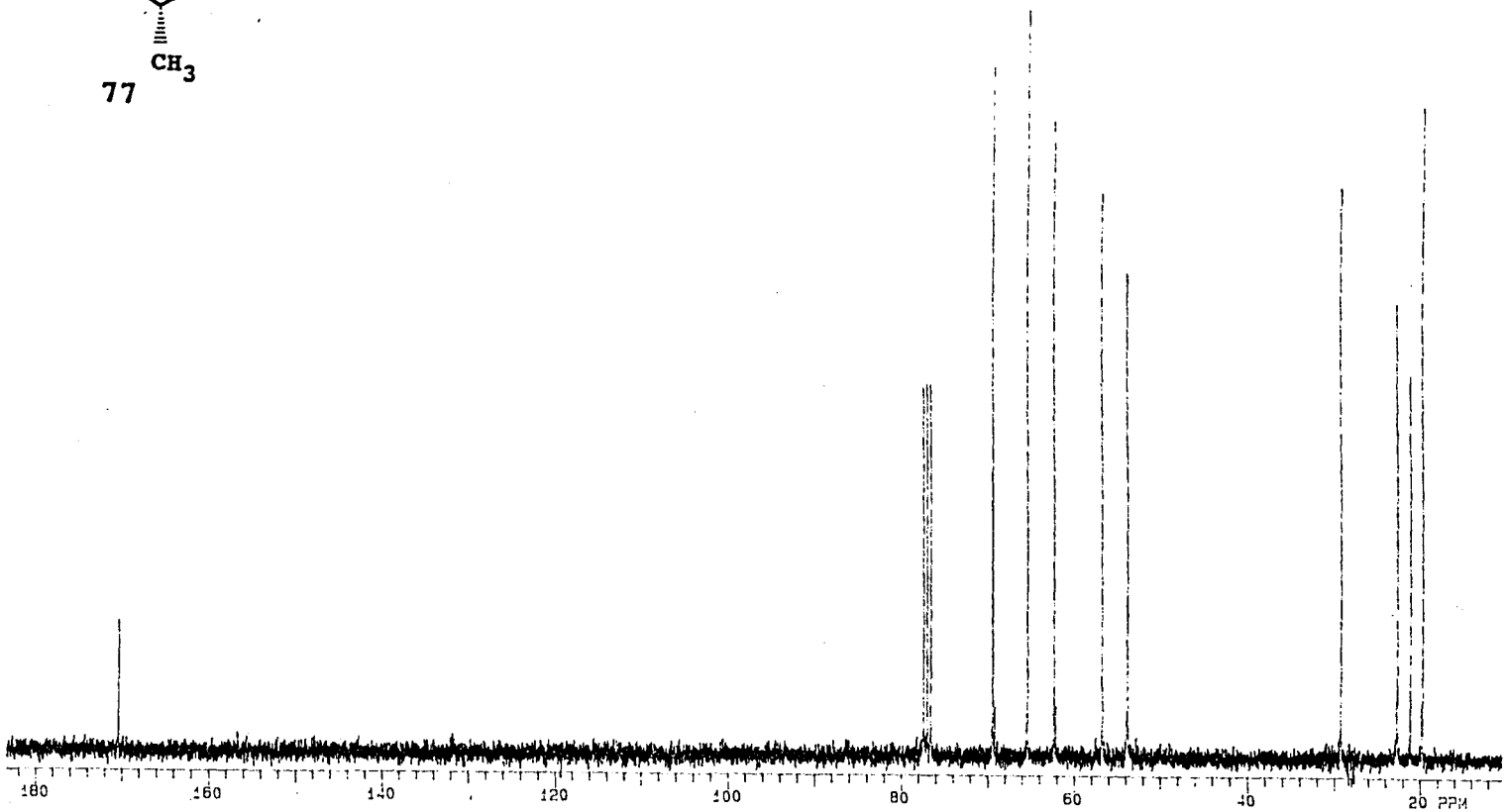
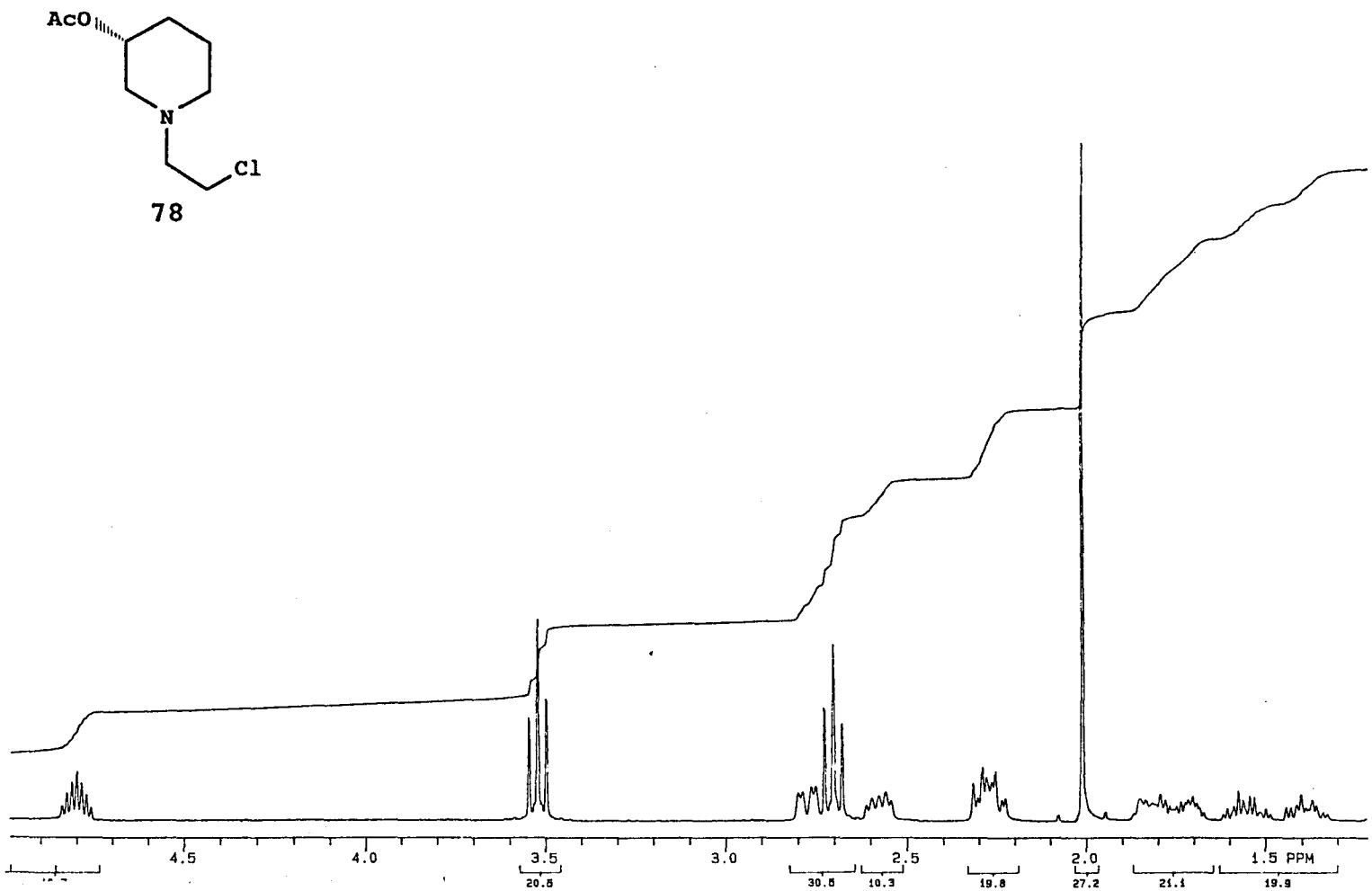
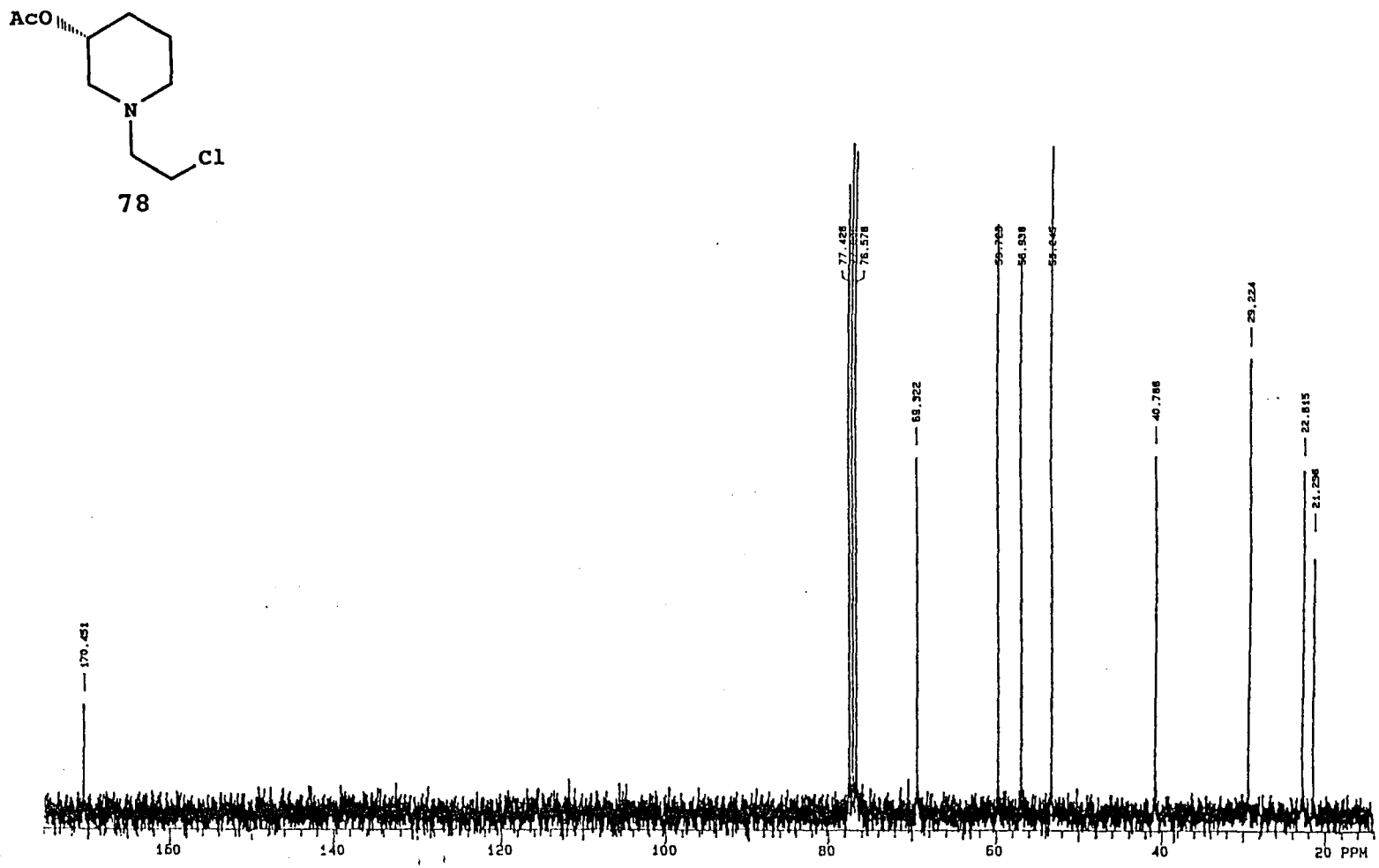


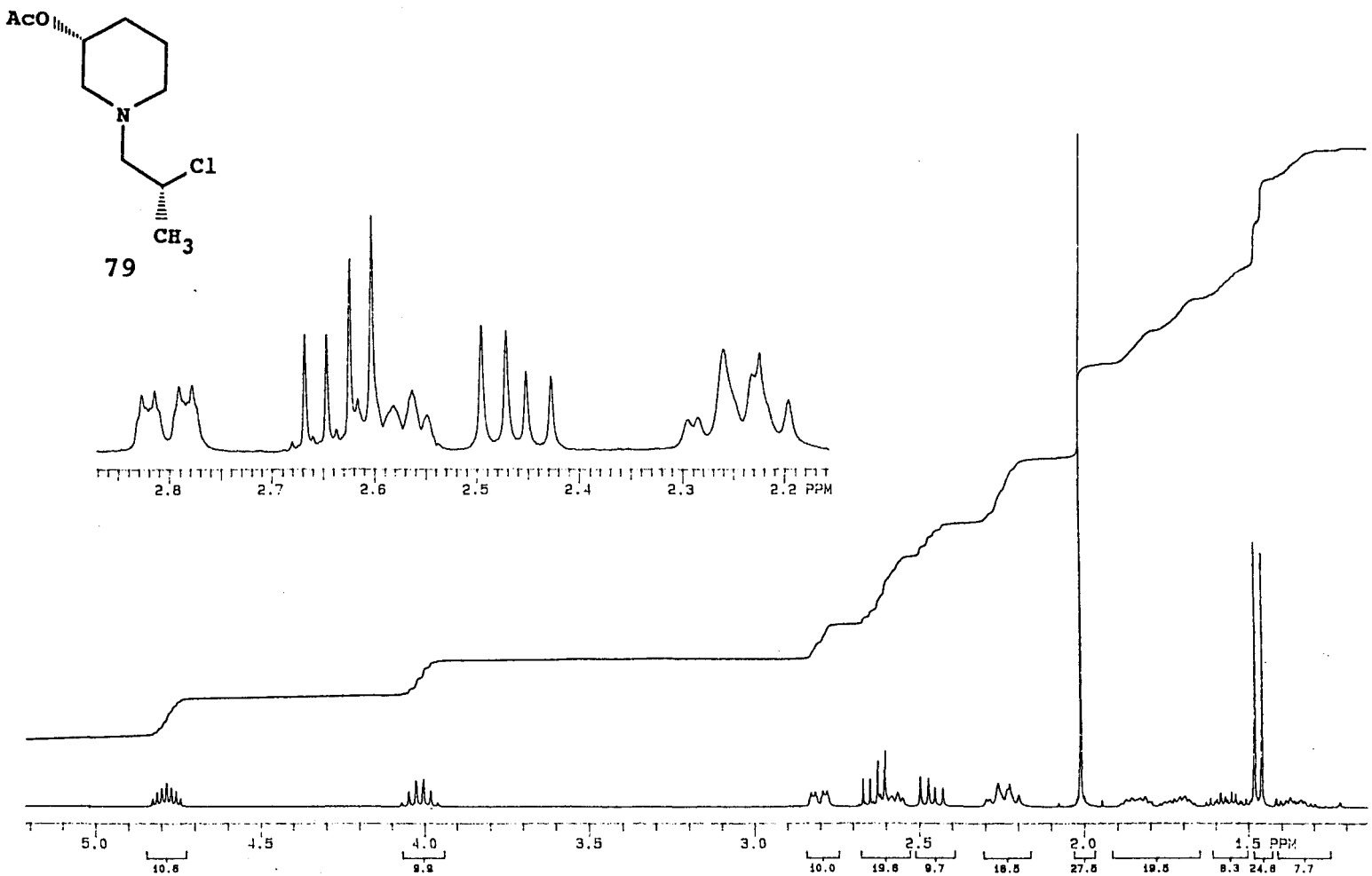
^{13}C NMR Spectrum of (R,R)-(-)-N-(2-Hydroxypropyl)-3-acetoxypiperidine (**77**)



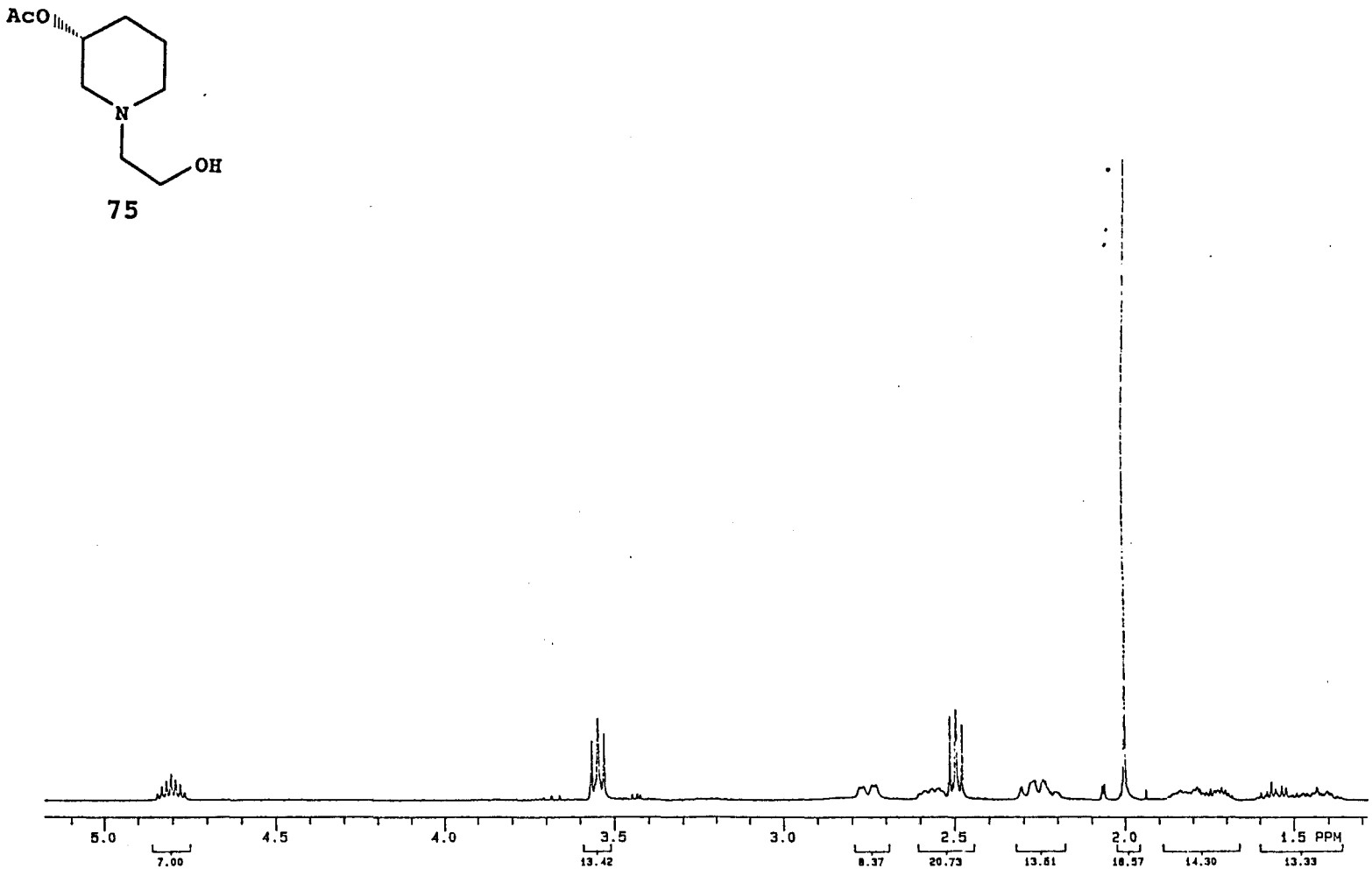
^{1}H NMR Spectrum of (R)-(+)-N-(2-Chloroethyl)-3-acetoxypiperidine (**78**)



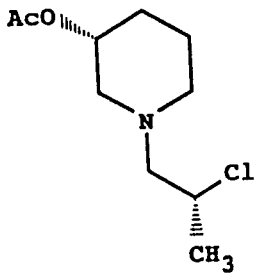
^{13}C NMR Spectrum of (R)-(+)-N-(2-Chloroethyl)-3-acetoxypiperidine (**78**)



^{1}H NMR Spectrum of (R,R)-(+)-N-(2-Chloropropyl)-3-acetoxypiperidine (**79**)



^{1}H NMR Spectrum of (R)-(+)-N-(2-Hydroxyethyl)-3-acetoxypiperidine (**75**)



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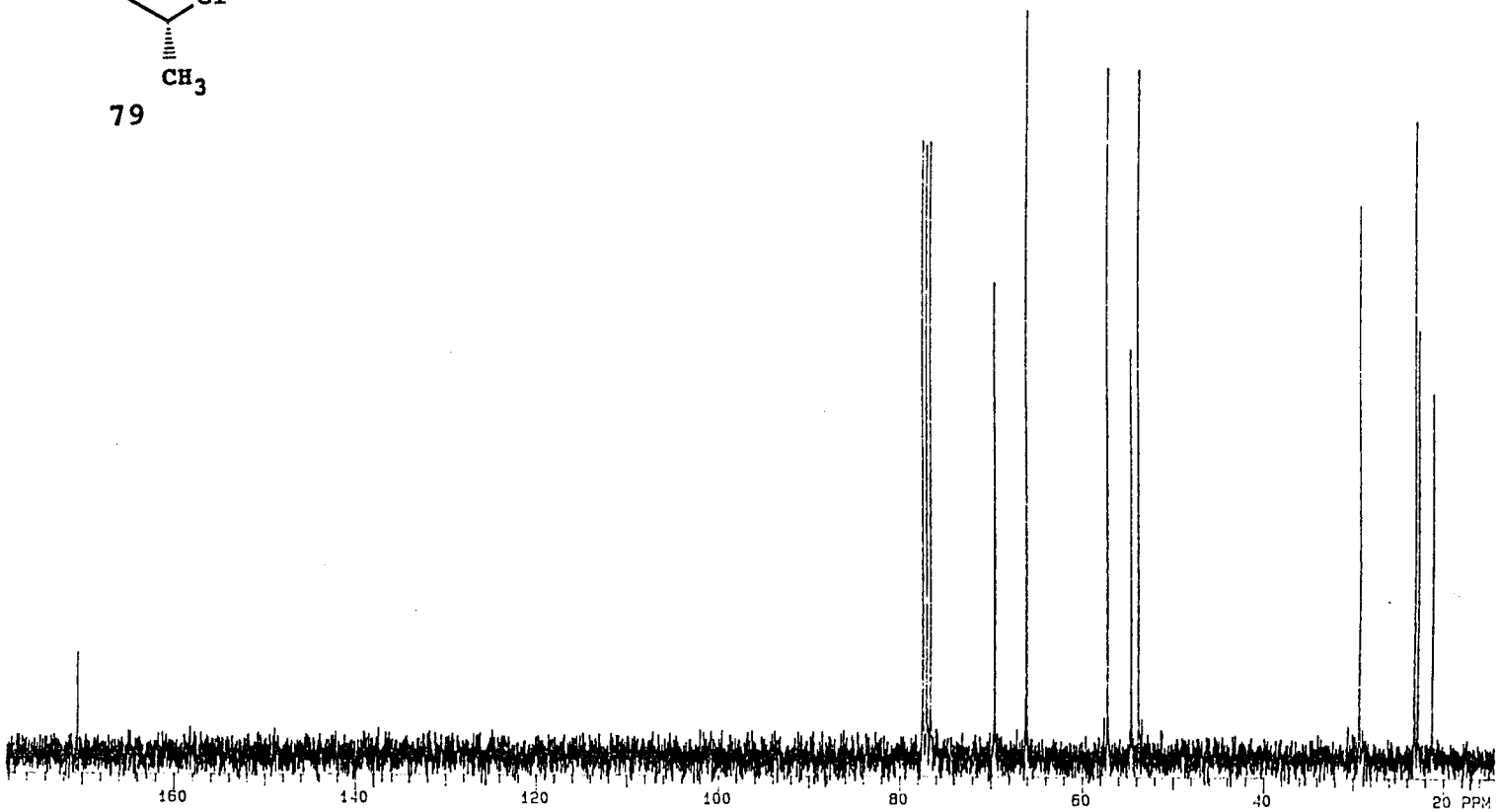
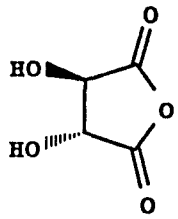


^{13}C NMR Spectrum of (R,R)-(+)-N-(2-Chloropropyl)-3-acetoxypiperidine (**79**)



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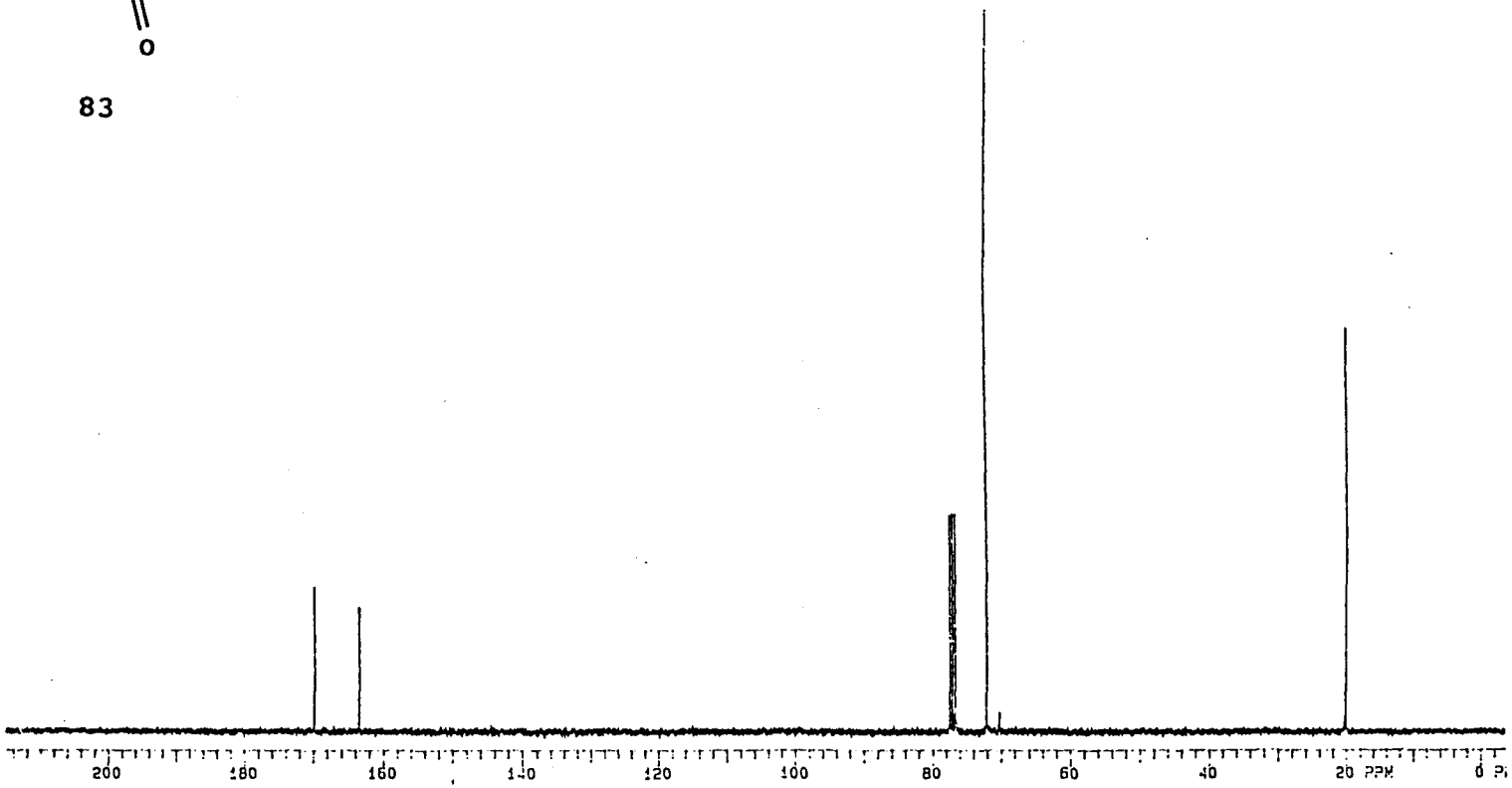
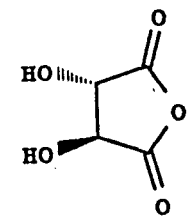


^{13}C NMR Spectrum of (R,R)-(+)-Diacetoxysuccinic anhydride (**83**)



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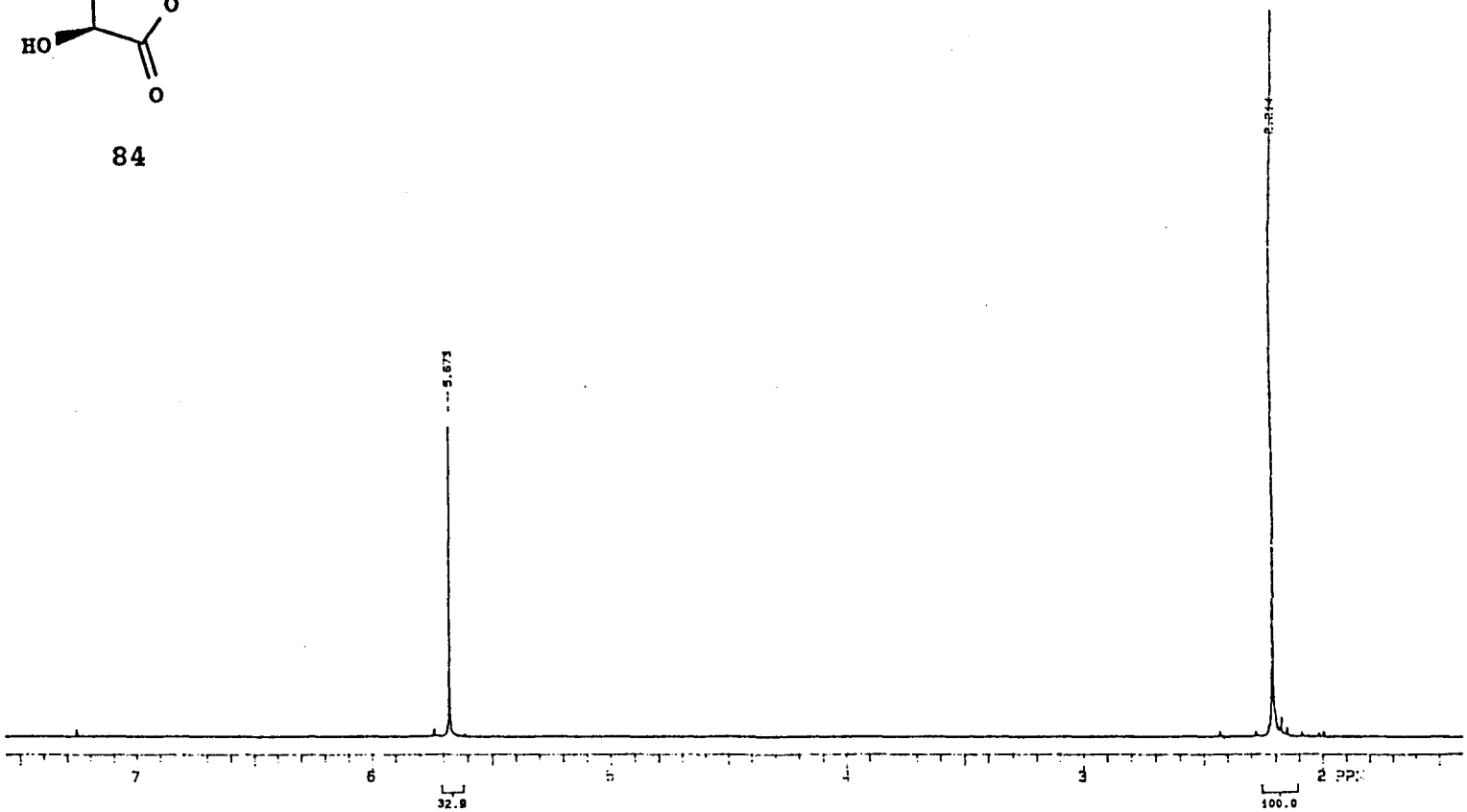
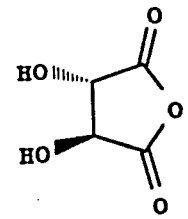


^{1}H NMR Spectrum of (S,S)-(-)-Diacetoxysuccinic anhydride (**84**)



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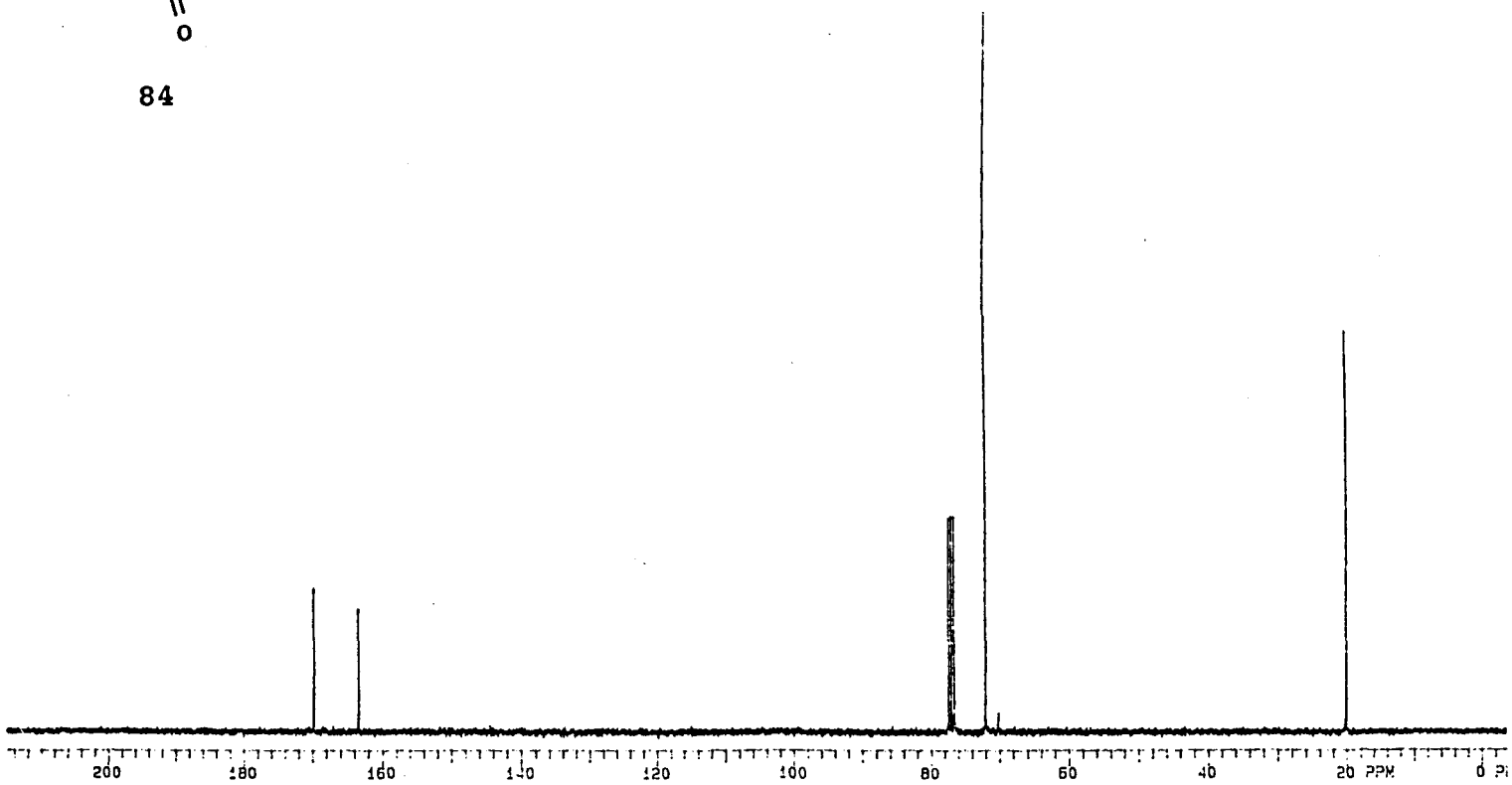
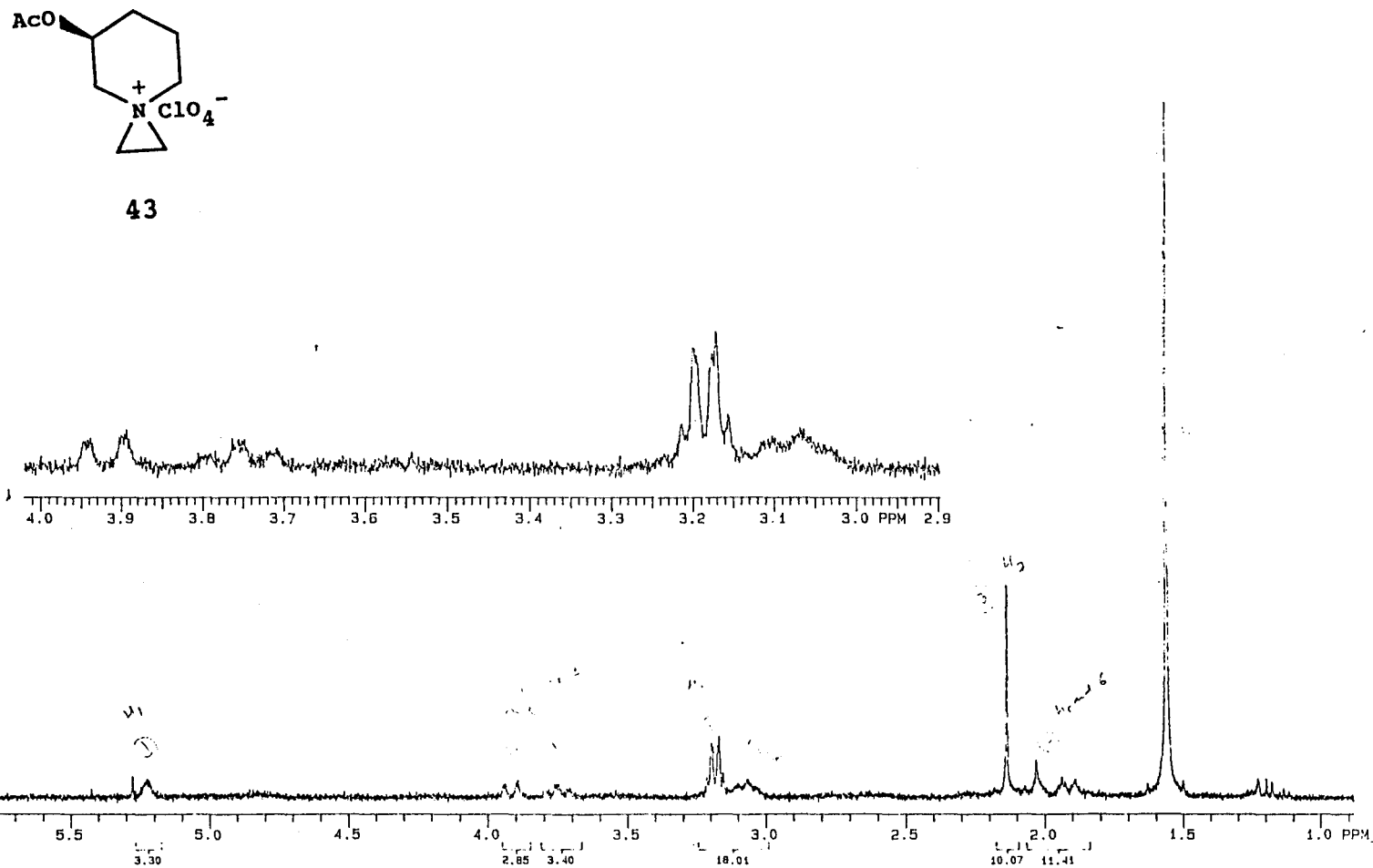


^{13}C NMR Spectrum of (S,S)-(-)-Diacetoxysuccinic anhydride (**84**)



^{1}H NMR Spectrum of (S)-5-Acetoxy-3-azoniaspiro[2.5]octane perchlorate (**31**)

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VITA

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Nam Huh was born on November 27, 1963, in Seoul, South Korea. After initial schooling in the Dong-Book Elementary School in Seoul, his secondary education was completed in February of 1982 at Kyung-Kee Senior High School in Seoul, South Korea. In March, he entered Seoul National University and received a Bachelor of Science in Chemistry in February, 1986. His senior thesis entitled "*Purification and study of enzyme specificity of α-galactase*" directed by Professor Myung Un Choi. In August, 1988 he began attending Loyola University of Chicago where he completed the requirement for the degree of Doctor of Philosophy in May of 1994. During his carrer at Loyola University of Chicago, he was awarded a Teaching Assitantship for General Chemistry during 1988-1989, and a Teaching Assitantship for Organic Chemistry during 1989-1990 and 1991-1992 academic years. During 1990-1991 and 1993-1994 academic years, he was awarded Research Assistantships and a Teaching Fellowship was awarded for 1992-1993 academic year. Research toward his Ph.D. dissertation "*Synthesis of Enantioenriched Piperidine-based, Spirocyclic Analogues of AF64A and Acetylcholine*" was guided by Professor

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Pulbications

(1) Chiral, Piperidine-Based Analogues of AF64A and Acetylcholine. Nam Huh, Charles M. Thompson *Bioorganic & Medicinal Chemistry Letters* **1992,** 2, 1551.

(2) Synthesis of Enantioenriched Piperidine-Based, Spirocyclic Analogues of AF64A and Acetylcholine (prep)

(3) Synthesis of Organophosphorus Compounds, Methyl-N-benzyl-O-(methylalkylphosphono)-serinoate hydrochloride (prep)

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the committee with reference to content and form.

The dissertation is, therefore, accepted in partial fulfillment of the requirements for the degree of doctor of philosophy.

April 13, 1994
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